PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Staley A. Brod

Serial No.: 10/801,277

Filed: March 16, 2004

For: Methods of Treating Rheumatoid Arthritis Using Orally Administered Type One Interferons

Group Art Unit: 1647

Examiner: Seharaseyon, Jegatheesan

Atty. Dkt. No.: CLFR:115USC1

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March 17, 2008
Date

David L. Parker

BRIEF ON APPEAL

This brief contains items under the following headings as required by 37 C.F.R. § 41.37 and M.P.E.P. § 1206:

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BRIEF ON APPEAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Appellant hereby submits this Appeal Brief. The date for filing this Brief is March 18, 2008. Should any additional fees become due under 37 C.F.R. §§ 1.16 to 1.21 for any reason relating to the enclosed materials, or should an overpayment be made, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski Deposit Account No. 50-1212/CLFR:115USC1.

I. REAL PARTY IN INTEREST

The real party in interest for this appeal is the assignee, Research Development Foundation.

II. RELATED APPEALS, INTERFERENCES, AND JUDICIAL PROCEEDINGS

There are no other appeals, interferences, or judicial proceedings related to this appeal.

III. STATUS OF CLAIMS

A. Total Number of Claims in Application

Claims 1-18 were filed with the Application. Concurrent with that filing, a preliminary amendment canceled claims 1-18 and added claims 19-30. Claims 19-30 are pending.

B. Current Status of Claims

All pending claims in the Application, *i.e.* claims 19-30, stand rejected.

C. Claims On Appeal

All pending claims in the Application, *i.e.* claims 19-30, are under appeal. The claims on appeal are reproduced in Appendix A.

IV. STATUS OF AMENDMENTS

Claims 19-30 were pending and under examination at the time the Final Rejection was mailed, September 18, 2007. Claim 23 was amended to correct a typographical error by amendment filed November 13, 2007. An Advisory Action issued January 24, 2008 denying entry of the amendment. After a call to the Examiner, the Examiner issued another Advisory

Action on February 29, 2008 allowing entry of the amendment but maintaining the rejection of the claims. Thus there are no un-entered amendments in this Application..

V. SUMMARY OF CLAIMED SUBJECT MATTER

Page and line numbers refer to the as-filed U.S. Application No. 10/801,277. The subject matter of the three independent claims currently appealed (*i.e.* claims 19, 23, and 27) is summarized below.

Independent claim 19 (See Claims Appendix) claims a method of preventing destructive joint disease (a later, destructive phase of rheumatoid arthritis disease; Specification, p.85 ll. 21-23) associated with rheumatoid arthritis (rheumatoid arthritis is a common chronic disorder involving the synovial membranes of multiple joints that is considered by most to be an autoimmune disease, *Id.* at p.4 ll. 6-8) (the American College of Rheumatology sets for criteria for the diagnosis of rheumatoid arthritis; *Id.* at p.77 ll. 17-18) in a human individual with an earlier stage of rheumatoid arthritis (a certain proportion of rheumatoid arthritis patients will eventually develop destructive joint disease; *Id.* at p.85 ll. 17-19) comprising: orally administering about 50 I.U./kg to about 25,000 I.U./kg of IFN-α to said individual (*Id.* at p.21 ll. 14-15; claims 4, 14, 18 as-filed) and immediately swallowing said IFN-α (*Id.* at p.78 ll.19-20).

Independent claim 23 (See Claims Appendix) claims a method of reducing inflammation (Specification, p.7 ll. 5-8) associated with rheumatoid arthritis (*Id.* at p.4 ll. 8-12) in a human individual with rheumatoid arthritis comprising: orally administering about 50 I.U./kg to about 25,000 I.U./kg of IFN-α to said individual (*Id.* at p.21 ll. 14-15; claims 4, 14, 18 as-filed); and immediately swallowing said IFN-α (*Id.* at p.78 ll.19-20).

Independent claim 27 (See Claims Appendix) claims a method of reducing a level of an interleukin (*See* Specification, Figure 23) in a human individual with rheumatoid arthritis, comprising: orally administering about 50 I.U./kg to about 25,000 I.U./kg of IFN-α to said

individual (*Id.* at p.21 ll. 14-15; claims 4, 14, 18 as-filed); and immediately swallowing said IFN-α (*Id.* at p.78 ll.19-20), thereby reducing the level of IL-1, IL-6, IL-8, or a combination thereof in said individual (*Id.*).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Were claims 19-30 improperly rejected under 35 U.S.C. § 112 ¶2 as indefinite?

B. Were claims 19-30 improperly rejected under 35 U.S.C. § 112 ¶1 as not enabled?

C. Were claims 19-26 improperly rejected under 35 U.S.C. § 103(a) over Shiozawa *et al.* ("Shiozawa") in view of Cummings, U.S. Patent No. 4,497,795 ('795) and Cummings U.S. Patent No. 5,019,382 ('382)

D. Were claims 27-30 improperly rejected under 35 U.S.C. § 103(a) over Shiozawa *et al.* ("Shiozawa") in view of Cummings, U.S. Patent No. 4,497,795 ('795) and Cummings U.S. Patent No. 5,019,382 ('382) and in further view of Aman *et al.* ("Aman") ?

VII. ARGUMENT

A. Properly Construed, The Claims are Definite

The Examiner states that claims 19-30 are rejected as indefinite under 35 U.S.C. §112 ¶2, however, only claims 20, 24 and 30 are specifically noted as unclear. The Examiner argues that it is unclear in these claims whether the amount of IFN-α administered is in units or international units, and whether the dosage administered is per kilogram of the subject, or total dose administered. However, the meaning of the claims is discernable when construed under correct principles, including: (A) the content of the particular application disclosure; (B) the teachings of the prior art; and (C) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. M.P.E.P. § 2173.02.

For example, construing the claims in the proper context of the Specification and prior art makes clear that claims 20, 24, and 30 refer to total dosage in international units (I.U.). Appellant's Specification recites that generally, interferon can be administered per the invention at a dosage of, "from about 50 I.U./kg to about 25,000 I.U./kg." Specification, p.21 ll. 14-15. Positive results were achieved when individual humans (regardless of kg per individual) each ingested 30,000 units total dosage of IFN-α. *Id.* at p.8 ll. 2-4; p.13 ll. 9-19. It is customary in the art to refer to interferon in terms of International Units ("I.U.") U.S. Patent Nos. 5,019,382 col. 3 ll. 45-52; 4,497,795 col. 4 ll. 59-63 (cited by Examiner). Further, claims 20, 24 and 30 each depend from claims that set forth dosage in International Units. Thus, a person of skill in the art reading the claims in the proper context would understand that "units" referred to in dependent claims 20, 24, and 30 refers to "international units."

Similarly, construing the claims in the proper context of the Specification and prior art makes clear that claims 20, 24, and 30 do not refer to units per kilogram. Appellant's Specification describes the rough equivalent dosage of 30,000 units for a 60 kg human to 10 units for a 20 mg mouse. *Specification*, p.56 ll.13-19. Claims 19, 23, and 27 require orally administering about 50 I.U./kg to about 25,000 I.U./kg of IFN-α. Administration of 30,000 units to a 60 kg human equals 500 units per kilogram, which clearly falls in the range required by the independent claims. In contrast, the Examiner's construction that 30,000 units could refer to 30,000 units/kg would result in improper dependent claims. For example, 30,000 units per 60 kg is 1,800, 000; clearly this is greater than the upper limit of the independent claims from which the rejected claims depend. All that is required for definiteness is that the claims be amenable to construction. *Honeywell Int'l v. International Trade Comm.*, 341 F.3d 1332, 1338-39 (Fed. Cir.

2003). Here, the claims are definite and discernable when properly analyzed. M.P.E.P. § 2173.02.

Appellants also note that the Examiner may have intended that Appellant to improve the clarity or precision of the claim language. However, attempting to accomplish this via rejection is expressly prohibited. *See* M.P.E.P. § 2173.02 ("[I]f the language used by applicant satisfies the statutory requirements of 35 U.S.C. 112, second paragraph, but the examiner merely wants the applicant to improve the clarity or precision of the language used, the claim must not be rejected."). Appellant's chosen language complies with the statutory requirements of 35 U.S.C §112 ¶2. Reversal of the rejection is respectfully requested.

B. The Claimed Invention is Enabled by the Specification

The Examiner has conceded enablement for (1) treating destructive joint disease associated with rheumatoid arthritis in an individual, (2) reducing inflammation associated with rheumatoid arthritis in an individual, and (3) reducing the level of interleukin in an individual with rheumatoid arthritis by oral administration of IFN-α . However, the Examiner rejects claims 19-22 for lack of enablement under 35 U.S.C. §112, ¶1, alleging that use of oral IFN-α to prevent destructive joint disease associated with rheumatoid arthritis in an individual is not enabled by the Specification because the Shiozawa *et al.* (1992) ("Shiozawa") reference and absence of guidance regarding patient selection and disease symptoms, provide the minimal requirement of providing reasons for uncertainty of enablement per *In re Bowen*, 492 F.2d 859, 862-63 181 USPQ 48, 51 (CCPA 1974). Final Action, p.4-6. As illustrated below, this is simply not the case. Shiozawa fails to provide the Examiner ***any*** basis to question enablement,

particularly in view of the guidance the Specification provides. Thus, the *Bowen* requirement is not met, the Examiner fails to carry his burden, and the rejection must be reversed.

1. The M.P.E.P. Requires a Reasonable, Sufficient Basis for Questioning Enablement

The rules require an Examiner making an enablement rejection to provide reasons (*i.e.* a basis) for questioning enablement, at a bare minimum. M.P.E.P § 2164.04; *In re Bowen*, 492 F.2d 859, 862-63 181 USPQ 48, 51 (CCPA 1974). However, even when providing only the least explanation the rules allow, the rules require the Examiner's reason or basis be sufficient and reasonable. *Id.* "The examiner has the initial burden to establish a ***reasonable basis*** to question the enablement provided...." *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (emphasis added). A proper rejection for failure to teach how to make and/or use is made when there is ***sufficient reason*** to doubt the statements in the specification that are relied upon for enabling support. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The Patent Office must back up its assertions with acceptable evidence or reasoning. *Id.* "[S]pecific, technical reasons are always required." M.P.E.P § 2164.04. Thus, the Examiner's burden is not discharged by providing "reasons," unless they are sufficient, reasonable, specific, and technical. Here, the Examiner's "reasons," are not sufficient, reasonable, specific, or technical. Thus the Examiner has failed to carry his burden.

2. What Shiozawa Does Not Teach Is Not Evidence of Non-enablement

The Examiner's first "reason" to question enablement is that Shiozawa teaches treatment of rheumatoid arthritis, but not the prevention of destructive joint disease. *See* Final Action, p.5. This position depends entirely upon what a reference ***does not teach***. If this logic is carried

forward, it means that any reference that does not teach an aspect of an applicant's invention, indicating it may have probative value under 35 U.S.C. §§ 102 or 103, automatically also provides a "reason" to question enablement under 35 U.S.C. § 112. Clearly, this is not specific or technical, and is an insufficient "reason" on its face.

Further, and specific to the instant facts, Shiozawa makes absolutely no mention of destructive joint disease. *See* Shiozawa (entire document). Shiozawa does not purport to be a complete authority on rheumatoid arthritis. *Id.* Shiozawa does not purport to describe the entire rheumatoid arthritis disease process and associated problems. *Id.* Thus, Shiozawa's failure to recognize and discuss destructive joint disease indicates nothing in relation to Appellant's claims. This void in Shiozawa is thus not a sufficient and reasonable basis, nor a specific and technical reason, to question whether Appellant's invention teaches prevention of destructive joint disease. The Examiner's burden is not met. M.P.E.P. § 2164.04

3. The Claims, Properly Construed, Are Enabled For The Identified Population

Next, the Examiner argues that (1) the specification does not identify a patient population selected for preventing destructive joint disease, and (2) if a patient population was identified with disease symptoms then onset of the disease has already taken place, thus disease prevention cannot be attained. Final Action, p.5. However, this "reason" also provides no basis to question enablement, and shows an apparent misunderstanding of Appellant's invention, specifically, the difference between destructive joint disease and rheumatoid arthritis ("RA").

RA patients can be identified that do not yet have destructive joint disease, and only prevention of destructive joint disease as claimed need be enabled. M.P.E.P. § 2164 ("The

invention that one skilled in the art must be enabled to make and use is that defined by the claim(s) of the particular application or patent."). Example 37 explains that a certain fraction of RA patients will develop destructive joint disease. Specification, p.85 ll. 17-19. Oral IFN-α therapy targets prevention of the ***destructive phase of RA*** in a non-toxic, patient-acceptable method. *Id.*, p.85 ll. 19-23. (emphasis added). Similarly, claim 1 is for, "a method of preventing ***destructive joint disease associated with rheumatoid arthritis*** in a human individual ***with an earlier stage of rheumatoid arthritis***...." Claim 1 (emphasis added). "Early treatment of RA with ingested IFN-α prevents or retards progression of RA...." Specification, p.86 ll. 11-13. Thus, Appellant has clearly identified a target patient population in the Claims as well as the Specification: RA sufferers who have not yet reached the destructive phase of RA, *i.e.*, destructive joint disease.

The Specification thus makes clear that other phases (besides destructive) of RA exist, and at least some phases or stages of RA occur before the onset of destructive joint disease. The Specification also teaches a patient having early stage rheumatoid arthritis but not suffering from destructive joint disease can be identified (*e.g.* by the ACR criteria for diagnosis of RA; Example 36) and treated with IFN-α to prevent the destructive phase from ever occurring. In view of this distinction between destructive joint disease and RA, and identification of potential patients ***in the claims***, Appellant has shown that no reason for questioning enablement on this basis exists and the Examiner's burden is clearly not met.

4. Adequate Examples and Guidance Are Provided

Furthermore, Example 36 demonstrates the results of an open label phase I study of orally ingested IFN-α in the treatment of patients with clinically stable RA. The compilation of results in Table 2 show that in 3 of the 4 patients treated there was substantial improvement in clinical disease indicia. Next, Example 37 describes, "a trend toward inhibition of CD3- and Con A-mediated IL-1, IL-6, and IL-8 secretion after eight weeks," of treatment with orally ingested IFN-α . Specification, p.84 ll.6-9. Considering a reduction of inflammatory cytokine production can prevent progression of RA, Example 37 clearly provides enabling support for Applicant's claims. *Id.*, p.85 ll. 2-16; p.86 ll. 11-13. One of skill in the art need only apply the teaching in Examples 36 and 37 specifically to early stage RA patients to practice the invention. Thus, Applicant has provided sufficient examples, guidance, and direction to enable the invention without undue experimentation. M.P.E.P. § 2164.01(a).

For all the foregoing reasons, Appellant submits that the Claims are enabled and respectfully requests reversal of the rejection of claims 19-22 under 35 U.S.C. §112 ¶1.

C. Under *KSR*, the Claims Are Not Obvious

The Examiner has rejected claims 19-26 as obvious over the combination of Shiozawa *et al.* ("Shiozawa") in view of Cummings, U.S. Patent No. 4,497,795 ('795) and Cummings U.S. Patent No. 5,019,382 ('382), and claims 27-30 as obvious over the combination of references above, further in view of Aman *et al.* ("Aman"). Specifically, the Examiner asserts that Shiozawa teaches administering IFN-α to treat rheumatoid arthritis, which allegedly will inherently treat destructive joint disease associated with rheumatoid arthritis; the '795 patent teaches oral administration and dosage; the '382 patent teaches conversion from I.U.to "units;"

and that the elements are expected to perform their expected functions to achieve their expected results when combined for a common known purpose. *See* Final Action, p.6-8. Aman is cited for teaching the reduction of interleukin-1 after interferon administration. Non-final Action dated 4/12/2007, p.9. However, as fully explained below, the Examiner has clearly failed to show that Appellant's claims are obvious in view of the prior art. M.P.E.P. § 2143.03

Graham v. John Deere Co., 383 U.S. 1, 148 USPQ 459 (1966), controls the consideration and determination of obviousness under 35 U.S.C. 103(a); *KSR Int'l Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1734-35, 167 L. Ed. 2d 705, 715 (U.S. 2007). The four factual inquires enunciated therein as a background for determining obviousness are: (1) determining the scope and contents of the prior art; (2) ascertaining the differences between the prior art and the claims in issue; (3) resolving the level of ordinary skill in the pertinent art; and (4) evaluating evidence of secondary considerations. In this case, neither the level of ordinary skill in the art nor secondary considerations are at issue. Thus, Appellant proceeds with the evaluation of (1) and (2).

1. *Graham* Factors: The Scope and Contents of the Prior Art

In order to assess the scope and content of the prior art properly, a thorough understanding of the invention must be acquired by studying Appellant's Claims and the Specification. M.P.E.P. § 2141. Claim 19 claims ***a method of preventing destructive joint disease*** associated with rheumatoid arthritis in a human ***with an earlier stage of rheumatoid arthritis*** comprising ***orally*** administering IFN-α to said individual. *See* Claim 19 (emphasis added). Similarly, Claim 23 claims a method of ***reducing inflammation associated with rheumatoid arthritis*** in a human individual with rheumatoid arthritis comprising ***orally***

administering IFN-α to said individual. *See* Claim 23 (emphasis added). And, Claim 27 claims a method of ***reducing a level of an interleukin*** in a human individual with rheumatoid arthritis, comprising ***orally*** administering IFN-α to said individual. *See* Claim 27 (emphasis added).

Shiozawa describes a 12-week study where intramuscular administration of α-interferon was given to RA patients. Shiozawa, p.405. Shiozawa subjectively assessed joint pain, tenderness, and swelling, and quantitatively tested serum calcium, alkaline phosphatase, parathyroid hormone, and osteocalcin. *Id.* at p.405, Tables I-III. However, as the Examiner conceded, Shiozawa, "does not teach the prevention of destructive joint disease...." Final Action, p. 7. Shiozawa does not consider or evaluate interleukin levels in an RA patient, or inflammation associated with RA. Further, the patients in Shiozawa were concomitantly administered anti-rheumatic treatments such as steroids and non-steroid anti-inflammatory drugs, with the α–interferon. Shiozawa, p.405, 407.

Regarding the Cummings patents, the Examiner asserts the '795 patent teaches oral administration and dosage of interferon and that the '382 patent teaches conversion from I.U. to "units." However, the conversion taught by the '382 patent is relevant only to ***that*** particular invention. *See* col. 3 ll. 45-56. The '382 patent describes units that cannot be directly converted to I.U.'s.

> As used herein, ***a "unit" of interferon*** (to be distinguished from "IU") ***shall mean the reciprocal of a dilution of interferon-containing material that, as determined by assay, inhibits one-half the number of plaques of a challenge virus,*** the challenge virus being the vesicular stomatitis virus ("VSV"). So quantified a "unit" of interferon is routinely found to be about one-tenth the quantity of interferon represented by one "IU." col. 3 ll. 45-56 (emphasis added).

The '795 patent teaches very low doses of orally administered interferon stimulate the appetite of chickens, cows, pigs, and guinea pigs. *See* Examples, '795 patent. Oral administration to humans is not taught, dosage for humans is not taught, and treatment of rheumatoid arthritis and/or related conditions is not taught. However, the '795 patent points out that in humans, interferon causes nausea and loss of appetite. *Id.* at col. 3 ll. 32-38. The '795 patent also states that it was widely believed that orally ingested interferon would not be biologically active. *Id.* at col. 3 ll. 1-2.

Lastly, Aman teaches the effect of IFN-α on certain tissue cultures. Aman, p.4147. The most dramatic result observed was suppression of hematopoiesis. *Id.* The Examiner cites Aman as teaching the reduction of interleukin-1 following the administration of α- interferon, but this is not entirely correct. Non-final Action, p.9. Aman teaches that in monocytic cell culture, IFN-α ***does not suppress*** IL-1, but in stromal cell culture, IFN-α ***significantly suppresses*** IL-1 production. *See* Aman, p.4148 col. 1 ¶2. Aman does not teach in vivo responses.

2. *Graham* Factors: Ascertaining the differences between the prior art and the claims in issue

When ascertaining the differences between the prior art and the claim at issue, both the invention and the prior art references as a whole must be considered, M.P.E.P. § 2141. Appellant's invention and the content of the prior art are thoroughly discussed above, taking into account the full teachings of each, as is required. *Id.*

a. The Differences Between the Claims and Prior Art Are Great

Shiozawa does not discuss destructive joint disease, interleukin levels in RA patients, or inflammation associated with RA. Thus, Shiozawa fails to teach what Appellant's claims, claim. M.P.E.P. § 2143.02. The Examiner argues that treatment of rheumatoid arthritis as described by Shiozawa will inherently treat destructive joint disease, however, ***this is not true.*** Because only a certain proportion of RA patients will develop destructive joint disease, treatment of RA does not *de facto* treat destructive joint disease. Specification, p.85 ll. 17-19. Because Shiozawa does not recognize destructive joint disease or a destructive phase of RA, Shiozawa cannot be said to even address the requirement in Appellant's Claim 19 that the preventative treatment be given to a patient with an earlier stage of RA. Obviousness cannot be predicated on what is not known at the time an invention is made, regardless of whether a certain feature is later deemed inherent. M.P.E.P. § 2143.02 *In re Rijckaert*, 9 F.2d 1531, 28 USPQ2d 1955 (Fed. Cir. 1993).

Furthermore, Shiozawa admits that the patients involved in the study were already taking disease modifying antirheumatic treatments. Shiozawa, p.407. Thus, Shiozawa ***cannot*** teach the treatment of anything that could also be treated by these other drugs (*i.e.* rheumatic disease and inflammation), because it is too tenuous an assumption that interferon was responsible for the reported results. Even Shiozawa recognizes this, making clear the need for further studies with a larger patient population ***not taking disease modifying antirheumatic treatments***. Shiozawa, p.407. In comparison, Appellant's claims reduce RA-associated inflammation and prevent RA-associated destructive joint disease. In sum, after comparing the differences between the cited art and the claims at issue, it becomes clear that the teaching of Shiozawa is irrelevant.

b. The Modifications Render the Prior Art Unsuitable

The '795 patent addresses appetite stimulation in chickens, cows, pigs, and guinea pigs. *See* Examples, '795 patent. There is no reason a person of skill in the art would read the '795 patent and believe oral administration of the described animal appetite stimulant could be applied to humans because the patent teaches that interferon has the opposite effect on humans. The '795 patent clearly states that interferon causes nausea and appetite loss when administered to people. '795 patent, col. 3 ll. 32-38. Thus, there is no suggestion to combine the human patient element of Shiozawa with the '795 patent. M.P.E.P. § 2143.01.

Alternatively, combining the oral administration aspect of the '795 patent with Shiozawa is similarly unsatisfactory. Shiozawa teaches only intramuscular administration of IFN-α, and teaches nothing about whether RA is amenable to therapy by oral IFN-α or what oral dosage would be effective. The '795 patent also explains that it was widely believed that the mammalian digestive environment would destroy interferon biological activity, and the levels taught in the '795 for appetite stimulation were too low to have other therapeutic effects. '795 patent, col. 3 ll. 1-2 and ll. 50-54. Thus, to the extent the '795 patent teachings could be extended to humans, a person of skill in the art would believe the resulting interferon levels would be too low for Shiozawa's purposes. M.P.E.P. § 2143.01.

c. Aman's Self Contradiction Eliminates Its Suggestive Power

According to the M.P.E.P., if teachings in the prior art conflict, the Examiner must weigh the suggestive power of each. M.P.E.P. § 2143.01. In this case, Aman teaches both that IFN-α (1) greatly suppresses IL-1 and (2)entirely fails to suppress IL-1, depending on the cell culture receiving the interferon. Accordingly, the Aman reference essentially discredits itself. M.P.E.P.

§ 2143.01. No person of skill in the art would read Aman's description of a phenomenon reported in certain tissue cultures, but reported as absent in others, and believe one of those results, but not the other, would translate into a drug therapy for humans suffering from RA that swallow their treatment rather than having it directly applied.

In sum, Appellants have shown that the differences between the prior art and the claimed invention are too great to be obvious to a person of skill in the art. *Dann v. Johnston*, 425, U.S. 219, 230, 189 USPQ 257, 261 (1976); M.P.E.P. § 2141. The claimed invention must be viewed as a whole, as is each prior art reference M.P.E.P. § 2141.02. When this is done, Appellants' claims are clearly not obvious.

3. No Proper Rationale Supporting Obviousness is Provided; The Examiner's Burden is Thus Not Met

Lastly, the Supreme Court has held that demonstrating the several claimed elements exist in the prior art is insufficient to find obviousness, rather, reasons for prompting a person of skill in the art to combine those claimed elements in the claimed fashion must be identified. *KSR Int'l Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741, 167 L. Ed. 2d 705, (U.S. 2007). Above Appellant shows the Examiner has not demonstrated the existence of the claimed elements in the prior art. Additionally, Appellant shows below that the Examiner has failed to articulate a reason for combining them, thus the burden of showing *prima facie* obviousness is not met. M.P.E.P. § 2142 ("The initial burden is on the examiner to provide some suggestion of the desirability of doing what the inventor has done.").

The Final Action cites M.P.E.P. § 2144.07 and states that the "prior art elements are expected to perform their expected functions to achieve their expected results when combined

for a common known purpose." *See* Final Action, p.6-8. Yet, no correlation of what element is supposedly performing what function resulting in what expected result, is provided. *Id.* Further, the M.P.E.P. recognizes that some degree of predictability such that an expectation of success is reasonable, is required when a proper rationale for finding obviousness is applied. M.P.E.P. § 2143.02. Both a reason to combine and the required degree of predictability are simply not present here.

First, Appellant reiterates that Shiozawa does not inherently teach treatment of the conditions treated by Appellant's method, and there is no reason to believe that IFN-α is responsible for whatever Shiowaza may treat considering the other medications taken by the studied patient population. Next, the '795 patent does not teach or suggest oral administration of interferon to humans with RA at modulatory levels, indicates it may not work in certain digestive systems, and describes interferon administered to humans as having side effects completely opposite of that intended for the use described therein. Thus, the element of treating destructive joint disease is not taught. The elements of treating destructive joint disease or inflammation associated with RA by IFN-α is not taught. The element of oral administration to a human is not taught. Oral administration to a non-ruminant mammal at levels to preserve modulatory effects is not taught. No known element performing a known function having an expected result is left! Thus, there is no proper reasoned basis justifying the legal conclusion of obviousness of claims 19-26. "Rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. at 1741.

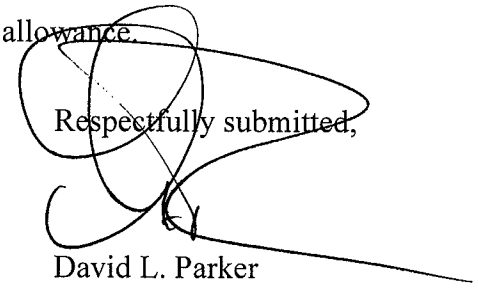
Similarly, for all the reasons just stated above, no proper rationale supporting obviousness is provided for claims 27-30, despite the inclusion of Aman. Aman is cited for teaching the reduction of interleukin-1 after interferon administration, but Aman's teaching is limited to tissue cultures. Depending on the culture type, the effect on of IFN-α on interleukin-1 varies from the dramatic to the un-measurable. Like Shiozawa, Aman admits the limits of its teaching, but Aman's are even further inapplicable as they are self-contradictory. The requisite level of predictable results and expectation of success needed to support an obviousness rejection of claims 27-30 is therefore also lacking.

Appellant has shown above that claims 19-26 are not obvious over the combination of Shiozawa in view of Cummings, '795 and Cummings '382; and claims 27-30 are not obvious over the combination of those references in further view of Aman. Reversal of the rejection of all claims under 35 U.S.C. § 103 is thus respectfully requested.

VIII. CONCLUSION

Appellant has shown above that all pending rejections are without merit and should be reversed. It is therefore respectfully requested that the Board overturn the rejections and recommend that this application proceed to allowance.

Respectfully submitted,



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Date: March 17, 2008

IX. CLAIMS APPENDIX

Appealed Claims:

19. A method of preventing destructive joint disease associated with rheumatoid arthritis in a human individual with an earlier stage of rheumatoid arthritis comprising: orally administering about 50 I.U./kg to about 25,000 I.U./kg of IFN-α to said individual; and immediately swallowing said IFN-α.

20. The method of claim 19, wherein about 30,000 units of IFN-α is orally administered.

21. The method of claim 19, wherein said IFN-α is administered every other day.

22. The method of claim 19, wherein said IFN-α is human recombinant interferon.

23. A method of reducing inflammation associated with rheumatoid arthritis in a human individual with rheumatoid arthritis comprising: orally administering about 50 I.U./kg to about 25,000 I.U./kg of IFN-α to said individual; and immediately swallowing said IFN-α.

24. The method of claim 23, wherein about 30,000 units of IFN-α is orally administered.

25. The method of claim 23, wherein said IFN-α is administered every other day.

26. The method of claim 23, wherein said IFN-α is human recombinant IFN-α.

27. A method of reducing a level of an interleukin in a human individual with rheumatoid arthritis, comprising: orally administering about 50 I.U./kg to about 25,000 I.U./kg of IFN-α to said individual; and immediately swallowing said IFN-α, thereby reducing the level of IL-1, IL-6, IL-8, or a combination thereof in said individual.

28. The method of claim 27, wherein about 30,000 units of IFN-α is orally administered.

29. The method of claim 27, wherein said IFN-α is administered every other day.

30. The method of claim 27, wherein said IFN-α is human recombinant IFN-α.

X. EVIDENCE APPENDIX

The following references were placed in the record by the Examiner by citation on April 12, 2007 in a form PTO-892

EXHIBIT A. Shiozawa *et al.* A preliminary study on the effect of alpha-interferon on the joint inflammation and serum calcium in rheumatoid arthritis (1992) British J. of Rheumatology, Vol. 31, pp.405-408.

EXHIBIT B. Aman *et al.* Regulation of cytokine expression by interferon-alpha in human bone marrow stromal cells; inhibition of hematopoietic growth factors and induction of interleukin-1 receptor antagonist (1994), Blood, Vol. 84 pp.4142-4250.

The following references were placed in the record by Applicant by citation on February 22, 2005 in a form PTO-1449, and considered by the Examiner on April 12, 2007.

EXHIBIT C. Cummings, U.S. Patent No. 4,497,795 (issued Feb 5, 1985).

EXHIBIT D. Cummings, U.S. Patent No. 5,019,382 (issued May 28, 1991).

Exhibit A

British Journal of Rheumatology 1992;31:405–408

PRELIMINARY REPORT

A PRELIMINARY STUDY ON THE EFFECT OF ALPHA-INTERFERON TREATMENT ON THE JOINT INFLAMMATION AND SERUM CALCIUM IN RHEUMATOID ARTHRITIS

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SUMMARY

A 12-week, double-blind controlled study comparing low dose α-interferon (5×10^5 iu, twice a week) and placebo was carried out to determine whether this drug has an immunomodulatory effect in rheumatoid arthritis. There were statistically significant improvements in the patients' joint score, C-reactive protein value and platelet count, without any side effects. Interferon-α caused an increase of serum calcium and a decrease of alkaline phosphatase. Thus, α-interferon requires further investigation as a possible additional useful treatment for rheumatoid arthritis.

KEY WORDS: Alpha-interferon, Rheumatoid arthritis (RA), Natural killer cell, Serum calcium.

CYTOKINES such as interleukin-1 (IL-1), IL-6 and tumour necrosis factor α play important roles in the pathogenesis of rheumatoid arthritis [1–6], and the immunomodulation of the action of these cytokines may influence the activity of the disease. We have previously found that circulating immunoreactive α-interferon [7], as well as the *in vitro* production of α-interferon [8–10], is significantly lower in almost all the patients with rheumatoid arthritis. Subsequent studies have shown that circulating α-interferon [11] is also low in Sjögren's syndrome [12], high in systemic lupus erythematosus [13–15] and normal in scleroderma and polymyositis. Circulating α-interferon is also low in the patients with hepatitis A [15] and in some patients with adult T-cell leukaemia [16], but normal in the measles patients who follow the typical clinical course with short-term recovery [17]. These findings suggest that the level of circulating α-interferon may vary in association with the host's ability to respond to exogenous antigens or viruses.

In order to examine the effect of supplementing the decreased amounts of α-interferon in patients with the objective of normalizing the host's response against antigens, we designed a 12-week, double-blind controlled trial comparing low dose of α-interferon and placebo. Alpha-interferon 5×10^5 iu administered twice a week, was without side effects. There were statistically significant improvements in the patients' joint score, C-reactive protein value and platelet count. Moreover, we found that administration of α-interferon increased serum calcium and decreased alkaline phosphatase levels.

PATIENTS AND METHODS

Study design

The study was a 12-week, double-blind trial comparing α-interferon and placebo in patients with rheumatoid arthritis fulfilling the ARA diagnostic criteria [18]. Patients were admitted into the study after their consent to the purpose of this investigation. Outpatients to the Kakogawa National Hospital, living approximately in the same district and with comparable lifestyles, were randomly assigned in two treatment groups (Table I). After 12 weeks, α-interferon was also started in the control group of patients.

Medication was unchanged from at least 3 months before the study, and fixed throughout the study period. The dosage combination drugs was: 25 mg of gold sodium thiomalate biweekly; 150 mg of D-penicillamine daily; 3 mg of prednisolone daily; 300 mg of thiopronin, a glutathione derivative, daily. All patients received non-steroidal anti-inflammatory drugs. The criteria for exclusion from the study were: (1) change of combination drugs; (2) intra-articular injection of corticosteroids; (3) admission to the hospital during the study period; (4) the appearance of significant side effects. Patients were given intramuscular injections of either α-interferon dissolved in saline or identical saline solution as placebo. Human leucocyte α-interferon [7] (5×10^5 iu, Ohtsuka Pharmaceutical Co. Ltd, Japan) was injected twice a week for 3 months.

Assessment

Clinical assessment was made by the same blinded physician (S.I.) before (baseline) and after 6 and 12 weeks of therapy. The joint count for tenderness on pressure and/or pain on motion for each of 60 diarthroidal joints and joint swelling for 58 joints were graded on a scale of 0, 1, 2 or 3 [19, 20]. Changes were considered significant when the score moved either between grade 0 and grades 1, 2 or 3, or between grade 1 and grade 3 [20].

Routine laboratory tests, including rheumatoid factor, serum immunoglobulins, complement, electrolytes, protein, albumin, total serum bilirubin, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), alkaline phosphatase (normal range; 26–10.0 K-AU), γ-glutamyl transpeptidase, lactic dehydrogenase, choline esterase, creatine kinase, amylase, blood urea nitrogen, creatinine, cholesterol, and triglycerides, were also performed. Serum calcium levels were corrected with or without the use of Payne's

Submitted 15 January; revised version accepted 29 April 1991.
Correspondence to Dr S. Shiozawa.

TABLE I
PATIENT PROFILE AT THE TIME OF ENTRY TO THE DOUBLE-BLIND STUDY

Variable	α-Interferon (n=17)	Placebo (n=14)
Age mean (range), years	54.0 (38–70)	54.8 (34–72)
Male/Female	4/13	3/11
Mean disease duration (years)	11.1	10.1
Stage 2	2	1
3	10	8
4	5	5
Class 2	11	9
3	6	5
Number of active joints, mean (range)	18.1 (6–30)	15.9 (6–28)
Blood sedimentation rate (mm/h)	60.9±24.2	62.0±27.0
C-Reactive protein (mg/dl)	3.2± 1.3	3.8± 1.4
Medications		
Gold thiomalate	5	3
Gold+corticosteroids	1	2
D-Penicillamine	4	2
D-Penicillamine+corticosteroids	3	2
Thiopronin	1	2
Thiopronin+corticosteroids	3	3

equation [21]. Serum osteocalcin and C-terminal parathyroid hormone (PTH) levels were quantitated by radioimmunoassay [22, 23].

Assay of natural killer activity

Natural killer cell activity against human erythroleukaemic K562 cell line was determined by a 4-h chromium-51 release assay [24], in which the result is expressed as the per cent natural killing activities.

Statistics

The difference in the means of selected variables between α-interferon and placebo groups was compared using the Student's t-test and Wilcoxon signed rank test, and expressed with mean ± SD.

RESULTS

All the patients completed the 3-month study (Table I). No patients experienced any side-effects such as fever. There were statistically significant improvements in the joint score, C-reactive protein value, and platelet count in the patients treated with α-interferon (Table II). The decrease in blood sedimentation rate observed in the α-interferon group was statistically insignificant. Laboratory tests including serum immunoglobulins, haemoglobin, white cell count, and lymphocyte count were all unchanged, except for a slight but significant increase of serum calcium and a decrease of alkaline phosphatase in the patients receiving α-interferon (Table III). There was no difference between two groups in the serum osteocalcin and C-terminal PTH levels. The natural killer cell activity against K562 cells showed no difference between the two groups (Fig. 1).

DISCUSSION

The result shows that α-interferon therapy improves certain inflammatory indices of rheumatoid arthritis such as the joint score, C-reactive protein value and platelet count. It appears that the low dose α-interferon treatment is safe and without side-effects when applied to rheumatoid arthritis. Although the blood sedimentation rate did not decrease significantly, the C-reactive protein level did fall. According to recent studies, although the blood sedimentation rate is an authorized indicator of the disease activity, the C-reactive protein value is also recommended as the most reliable, external and objective measure of rheumatoid inflammation [20]. Since the blood sedimentation rate is inherently subject to variation because it is visually read, the C-reactive protein value may be superior for evaluating the slight difference in results in such a comparative study as this.

Since the low dose of α-interferon used in this study does not inhibit cell proliferation [25], α-interferon must have exerted its effect by a different mechanism than those of other antirheumatic, cytotoxic agents. This may explain why the effect of α-interferon was not comparable to that of cytotoxic drugs that act directly on the proliferative phase of rheumatoid synovitis. Previous studies have shown that α-interferon acts on the cells primarily responsible for the host's front-line defence against invading organisms [26, 27]: α-interferon is necessary for continued activation of natural killer cells [28, 29]. It is possible that the decrease of α-interferon and resulting dysfunction of these cells lead to abnormal recognition of the pathogenic antigens, if the immune response against antigen plays a major role in the perpetuation of synovitis [30]. Since the synovial lining cells possess both mesenchymal [31, 32] and macrophage-like [33, 34] character, α-interferon may

TABLE II
RESULT OF α-INTERFERON THERAPY ON RHEUMATOID ARTHRITIS

	α-Interferon (n=17)					Placebo (n=14)		
	0	6		12		0	6	12 (Weeks)
Joint count								
Pain	10.3± 6.8*	8.4± 4.4		8.8± 6.4		8.4± 5.0	9.8± 6.5	9.1± 5.4
Swelling	14.8± 5.9	12.1± 5.6	P=0.005	12.3± 7.2		14.1± 6.3	13.9± 8.7	12.2± 8.6
Total index	18.1± 6.1	15.3± 6.1	P=0.007 (P=0.05)	13.3± 6.3	P=0.05 (P=0.05)	15.9± 6.2	16.4± 8.5	15.0± 8.4
Blood sedimentation rate (mm/h)	60.9±22.3	57.0±31.2		57.8±34.4		62.0±27.1	57.2±31.8	64.5±29.8
C-reactive protein (mg/dl)	3.2± 1.3	2.2± 1.7	P=0.006	1.9± 1.2	P=0.002	3.8± 1.8	3.8± 1.2	3.1± 1.3
Platelet count (10^4/mm^3)	32.7± 8.3	28.6± 7.1	P=0.02	29.5± 6.6		32.7±11.3	35.0±13.7	35.8±10.3

Statistics by the Student's t-test, and Wilcoxon signed rank test in parenthesis.
*Mean±SD.

TABLE III
LABORATORY TESTS BEFORE AND AFTER THE α-INTERFERON TREATMENT

	α-Interferon (n=31)		Placebo (n=14)	
	Before	After	Before	After
Serum calcium (mEq/l)	4.49±0.20†	4.56±0.25*	4.48±0.22	4.53±0.20
Corrected serum calcium (mEq/l)	4.40±0.20	4.48±0.17***	4.46±0.12	4.45±0.14
Alkaline phosphatase (K-AU)	9.92±3.67	9.01±2.51**	9.28±2.44	9.83±2.31
Parathyroid hormone (ng/ml)	0.62±0.42	0.61±0.45	0.63±0.23	0.54±0.30
Osteocalcin (ng/ml)	2.31±1.27	2.45±1.18	3.59±1.94	2.66±1.13

*P<0.05, **P<0.025, ***P<0.01 by the paired Student's t-test.
†Mean±SD.

act directly on the synovial cells to modulate their function.

It was noted in this study that α-interferon increased serum calcium and simultaneously decreased serum alkaline phosphatase. The decrease of alkaline phosphatase suggests the possibility that α-interferon inhibits the differentiation of osteoblasts. This is in line with the inhibitory action of γ-interferon on the differentiation and/or proliferation of osteoblasts [35–37]. Since recent studies have shown that the osteoblast is the major regulator of bone resorption [38], it is possible that α-interferon inhibits rheumatoid bone resorption by inhibiting the osteoblast. Alpha-interferon indeed inhibits PTH-stimulated bone resorption *in vitro* [39]. The increase of serum calcium by α-interferon treatment suggests either that α-interferon increases the intestinal absorption of calcium, that α-interferon enhances osteoclastic bone resorption, or that α-interferon inhibits the utilization of calcium within the bone by inactivating both osteoblasts and osteoclasts. It is unlikely that α-interferon changes the renal handling of calcium, because serum parathyroid hormone levels remain unchanged in patients receiving α-interferon.

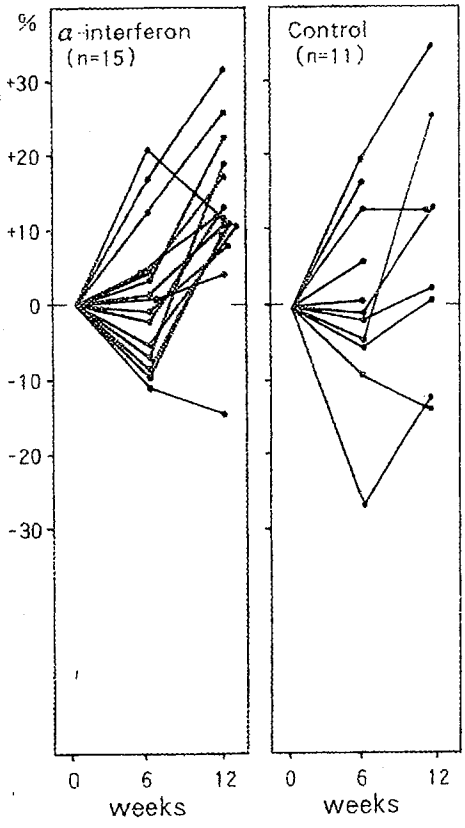

FIG. 1.—Natural killer cell activity against K562 cells.

In summary, the low dose α-interferon treatment improved the joint score, C-reactive protein value and platelet count in patients with rheumatoid arthritis, without significant side-effects. Because of its possible unique mode of action and effects on bone metabolism, α-interferon requires further investigation as a useful additional form of the treatment of rheumatoid arthritis. A further study enrolling larger numbers of patients without concomitant disease modifying antirheumatic treatment might generate additional information on the effect of α-interferon.

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Exhibit B

Regulation of Cytokine Expression by Interferon-α in Human Bone Marrow Stromal Cells: Inhibition of Hematopoietic Growth Factors and Induction of Interleukin-1 Receptor Antagonist

By M. Javad Aman, Ulrich Keller, Günther Derigs, Mansour Mohamadzadeh, Christoph Huber, and Christian Peschel

We investigated the effects of interferon-α (IFN-α) on the expression of cytokines by human bone marrow stromal cells. Production of granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-CSF (G-CSF), and interleukin-1β (IL-1β) in stromal cell layers was induced by incubation with IL-1α, tumor necrosis factor (TNF), or lipopolysaccharide (LPS). Addition of IFN-α to such stimulated cultures resulted in a strong downregulation of mRNA expression of GM-CSF and IL-1β. Similarly, the protein levels of GM-CSF and IL-1β were significantly reduced by IFN-α, whereas G-CSF production was only moderately inhibited. In contrast, IFN-α markedly stimulated the production of IL-1 receptor antagonist (IL-1RA) by stromal cells. The inhibition of cytokine expression resulted in a reduced hematopoietic activity of stromal cells, indicated by a reduced proliferation of the factor dependent cell line MO7e on IFN-α–treated stromal cells. In the presence of cycloheximide (CHX), IFN-α failed to inhibit IL-1 mRNA expression, whereas the regulation of GM-CSF and IL-1RA by IFN-α was not affected. Our results indicate that the myelosuppressive effects of IFN-α, as observed in therapeutic applications or associated with viral infections, are, in part, indirectly mediated by inhibition of the paracrine production of hematopoietic growth factors.

LONG-TERM HEMATOPOIESIS in vitro is supported by nonhematopoietic connective tissue cells of marrow origin collectively referred to as stromal cells.[1] For stem cell renewal and sustained hematopoiesis, a complex cellular interaction with stromal cells is required, which includes yet poorly defined signals mediated by cellular and matrix structures and the production of humoral factors by stromal cells.[2-7] Hematopoietic growth factors are detected in stromal cell lines and Dexter type cultures of murine or human origin on stimulation with cytokines, such as interleukin-1 (IL-1), or lipopolysaccharide (LPS).[8-11] However, granulocyte-macrophage colony-stimulating factor (GM-CSF) is also constitutively produced by stromal cells in small, but biologically relevant, amounts.[12,13] Novel cytokines, including IL-7, IL-11, and stem cell factor, have been detected as products of stromal cells.[14-16] Negative regulators, such as transforming growth factor-β (TGF-β) and tumor necrosis factor (TNF), are also produced by bone marrow stromal cells to maintain homeostasis of the hematopoietic system in vitro, and apparently, in vivo.[17,18] In addition to stromal cells, the cellular composition of the bone marrow microenvironment comprises immune regulatory cells, including monocytes and T lymphocytes. The role of many cytokines produced by those immune cells in the regulated expression of factors with stimulatory or inhibitory effects on hematopoiesis remains to be determined.

Interferon-α (IFN-α) has a well-defined beneficial role in the treatment of myeloproliferative diseases, including chronic myelogenous leukemia (CML),[19] polycythemia vera,[20] and essential thrombocythemia.[21] Although the mechanism of a selective suppression of the malignant clone by IFN-α in subpopulations of CML patients is unclear, cytogenetic responses are clearly associated with the more frequent hematologic responses of CML patients,[19] which can be explained by the myelosuppressive effect of IFN. Direct antiproliferative effects of IFNs on hematopoietic progenitors are well known.[22] However, the regulation of cytokine cascades by type-I IFNs, as recently observed for IL-8[23] and IL-1 receptor antagonist (IL-1RA),[24] might contribute to the role of IFN as an inhibitory agent of hematopoiesis.

In this study, we used human stromal cell cultures to evaluate the effects of IFN-α on the expression of cytokines that are involved in the regulation of hematopoiesis. We demonstrate that IFN-α substantially inhibits the production of cytokines with stimulatory activity on hematopoietic progenitors and additionally counteracts IL-1 effects by inducing IL-1RA. These results indicate that the myelosuppressive effects of IFN-α are, at least, in part mediated by modulating the expression of hematopoietic growth factors.

MATERIALS AND METHODS

Materials. rh-IFN-α 2b with a specific activity of 1.8×10^8 U/mg was obtained from Essex Pharma (München, Germany). rh-GM-CSF was kindly provided by Sandoz AG (Nürnberg, Germany), rh-IL-1α by Hoffmann La Roche (Nutley, NJ), and rh-TNF-α (6.6×10^6 U/mg) by Knoll AG (Ludwigshafen, Germany). LPS was purchased from Sigma Chemicals (Deisenhofen, Germany), actinomycin-D and cycloheximide (CHX) from Merck, Inc (Darmstadt, Germany), ^{3}H-thymidine (20 Ci/mmol) from DuPont (Bad Homburg, Germany), monoclonal anti-huGM-CSF antibody from Boehringer (Mannheim, Germany) and α^{32}P-labeled nucleotides from Amersham Buchler (Braunschweig, Germany).

Stromal cell culture. Bone marrow stromal cell cultures were essentially obtained as described previously[25] with some modifications.[23] Briefly, bone marrow mononuclear cells, separated by centrifugation over Ficoll-Hypaque, were incubated at a cell density of 1×10^6/mL in 25 cm^2 tissue flasks at 33°C in culture medium consisting of RPMI 1640 supplemented with 10% fetal calf serum

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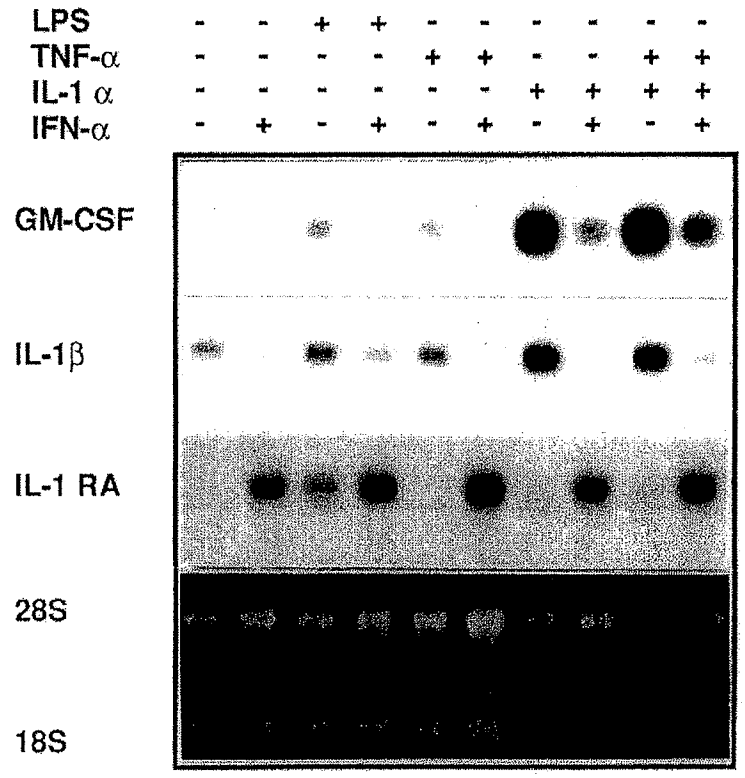

Fig 1. Effect of IFN-α on cytokine mRNA expression. Confluent secondary stromal cultures were incubated in medium for 6 hours with the indicated factors. Total RNA was prepared and subjected to Northern-blot analysis with cDNA probes for GM-CSF, IL-1β, and IL-1RA. Concentrations used: IFN-α 1,000 U/mL, TNF-α 500 U/mL, LPS 10 mg/mL, and IL-1α 100 U/mL. Data are representative for six experiments with stromal cells from different donors. The lower part of the panel shows the 18S and 28S rRNA from ethidium bromide-stained gel as loading control.

(FCS), 10% horse serum, 1.0 μmol/L hydrocortisone (Sigma Chemicals), and the additives as described.[23] In weekly intervals, cultures were fed by replacing 75% of the culture medium. When cultures were covered more than 80% by adherent cell, the primary cultures were treated with trypsin-EDTA (Biochrom, Berlin, Germany). Detached stromal cells were pooled and expanded in new culture flasks at a surface ratio of 1:5 in 75 cm^2 tissue flasks. The adherent cells were incubated under the same culture conditions until the cultures became confluent again. By this culture method, homogenous stromal cell layers were obtained that were morphologically and functionally comparable in different culture flasks. Hydrocortisone was removed from these cultures at least 3 days before use for RNA and protein analysis. The viability of stromal cells after IFN treatment for 24 hours was evaluated by counting the viable cells after detachment by trypsin/EDTA and staining with trypan blue. IFN-α was shown to have no effect on the viability of stromal cells under these conditions. GM-CSF, granulocyte-colony-stimulating factor (G-CSF), and IL-1RA were measured in the culture supernatants of stromal cells after incubation for 24 hours with various factors, as indicated in the Results section. Cell-associated IL-1β was measured in cell lysates of the same cultures. For preparation of cell lysates, stromal cells were washed three times with phosphate-buffered saline (PBS) and scraped from the culture flasks. The detached cells were resuspended in 1.0 mL supplemented RPMI 1640 and sonified two times for 10 seconds using an ultrasound sonifier. Cell debris was removed by centrifugation, and the supernatant was used for IL-1β measurement. For RNA analysis, the factors as indicated in the Results section were added to parallel culture flasks from the same donor. After 6 hours the cells were lysed directly in the culture flask with guanidium-isothiocyanate lysis buffer and processed as described below. The content of a 75 cm^2 tissue flask yielded 10 to 20 μg total RNA.

Northern blot analysis. Total cytoplasmic RNA was prepared using the single step method of guanidinium/phenol-chloroform extraction as described previously.[26] Ten to 15 μg RNA (depending on the least yield obtained in each experiment) were subjected to electrophoresis on a 1% agarose-formaldehyde gel and transferred onto nylon membrane (Hybond-N, Amersham Buchler). Blots were hybridized to α^{32}P-labeled cDNA probes using random primer DNA labeling kit (Boehringer), washed, and exposed to Cronex-4 autoradiography films (DuPont) at −70°C. hu-GM-CSF cDNA, an 800-bp *Eco*RI fragment cloned into P91023(B)-vector, and hu-IL-1β cDNA, a 600-bp *Bam*HI fragment in YEpsec1-vector, were obtained from American Type Culture Collection (Rockville, MD). A cDNA probe for IL-1RA was prepared by polymerase chain reaction (PCR) amplification from RNA obtained from peripheral blood mononuclear cells (PBMNC) after reverse transcription using specific primer for IL-1 RA, which resulted in a 492-bp fragment. Primer sequences: sense: 5′ATGGAAATCTGCAGAGGCCTC3′, antisense: 5′TTCGTCAGGCATATTGGTGAGGCTGAC3′.[27] Reverse transcription and PCR amplification were carried out as previously described.[28] Enzyme-linked immunosorbent assay (ELISA): Concentrations of GM-CSF, G-CSF, IL-1RA, and IL-1β were measured using Quantikine Human Cytokine Immunoassays (R & D Systems, Minneapolis, MN) according to manufacturer's instructions. Sensitivity of the tests was as follows: GM-CSF 7.8 pg/mL, G-CSF 31 pg/mL, IL-1β 3.9 pg/mL, and IL-1RA 31 pg/mL.

Isolation of human foreskin fibroblasts. Foreskin (obtained from the Division of Pediatrics, University of Mainz) was kept in 10% FCS/PBS for 30 minutes and subsequently washed in PBS. Epidermis was isolated from the foreskin using fine forceps and subsequently incubated in PBS supplemented with 2.5 U/mL dispase type II (Boehringer) at 37°C for 3 hours. After removal of dispase solution, foreskin was rinsed three times with PBS and incubated at 37°C in PBS containing 0.2% (wt/vol) trypsin type III/0.1% EDTA (Sigma) with gentle agitation for 15 minutes. A single-cell suspen-

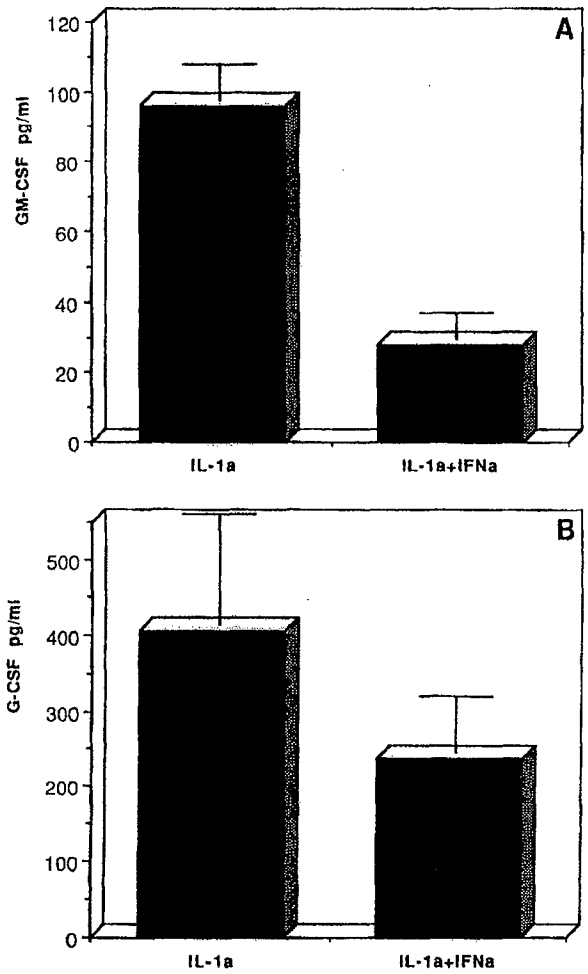

Fig 2. Effect of IFN-α on the secretion of GM-CSF and G-CSF. Confluent secondary stromal cultures were incubated in medium containing 100 U/mL IL-1α with or without 1,000 U/mL IFN-α for 24 hours, and the cytokine concentration in the supernatants was assessed using specific ELISAs. Single experiments were carried out in triplicate, and data from five experiments are summarized as mean ± SEM.

sion was obtained by filtering the suspension through a stainless steel mesh. The residual dermal fragments were rinsed once with PBS/10% FCS and discarded. Cells were sedimented at 230*g* for 10 minutes at 4°C and washed twice with PBS/10% FCS. Cells were incubated at 10^6/mL in Iscove's medium. Subconfluent first passage fibroblasts were used for RNA experiments. The content of a 75 cm^2 tissue flask yielded 10 to 20 μg total RNA.

Proliferation assay. MO7E cells were incubated in 96-well flat bottom microtiter plates either alone or on preformed stromal cell monolayers in supplemented RPMI 1640 + 10% FCS at 37°C/5% CO_2 with the factors indicated in the Results section. After 3 days, 1 μCi/well ^{3}H-thymidine was added. Cells were harvested after 16 hours in a PHD-Cell Harvester (Dunn Labortechnik, Asbach, Germany) and thymidine incorporation was quantified in a β-counter (Beckmann Munich, Germany).

RESULTS

Regulation of cytokine mRNA expression by IFN-α. Expression of cytokine mRNA was examined in stromal cell cultures derived from five normal bone marrow samples. Multiple stromal cell cultures of individual donors were pooled after trypsinization and expanded in secondary cultures, to avoid heterogeneity of the cellular components and cell density. In such homogenous cultures, the mRNA expression of GM-CSF, IL-1β, and IL-1RA was examined in unstimulated stromal cells after addition of various stimuli. Low levels of constitutive expression of GM-CSF and IL-1β were observed in two samples, whereas IL-1RA expression was never detected. The addition of IL-1α, LPS, or TNF-α to stromal cell cultures induced the expression of

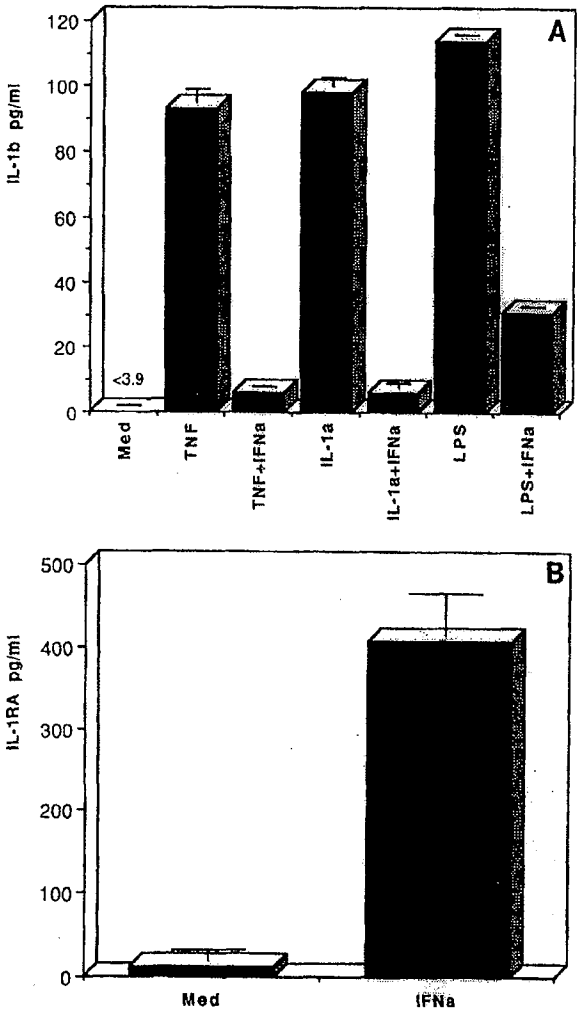

Fig 3. Effects of IFN-α on the production of cell bound IL-1β and secreted IL-1RA. For IL-1β measurement, confluent secondary stromal cultures were incubated in medium with the indicated factors for 24 hours. Cells were lysed in 1 mL medium by sonifying, and IL-1β concentration in the lysates was assessed using a specific ELISA. IL-1b data represent mean ± SEM from one typical experiment out of five, carried out in triplicate. IL-1RA was measured in supernatants of stromal cultures incubated for 24 hours in medium with or without IFN-α. Single experiments were carried out in triplicate and summarized data from five experiments are presented as mean ± SEM. Concentrations used were the same as indicated in the legend to Fig 1.

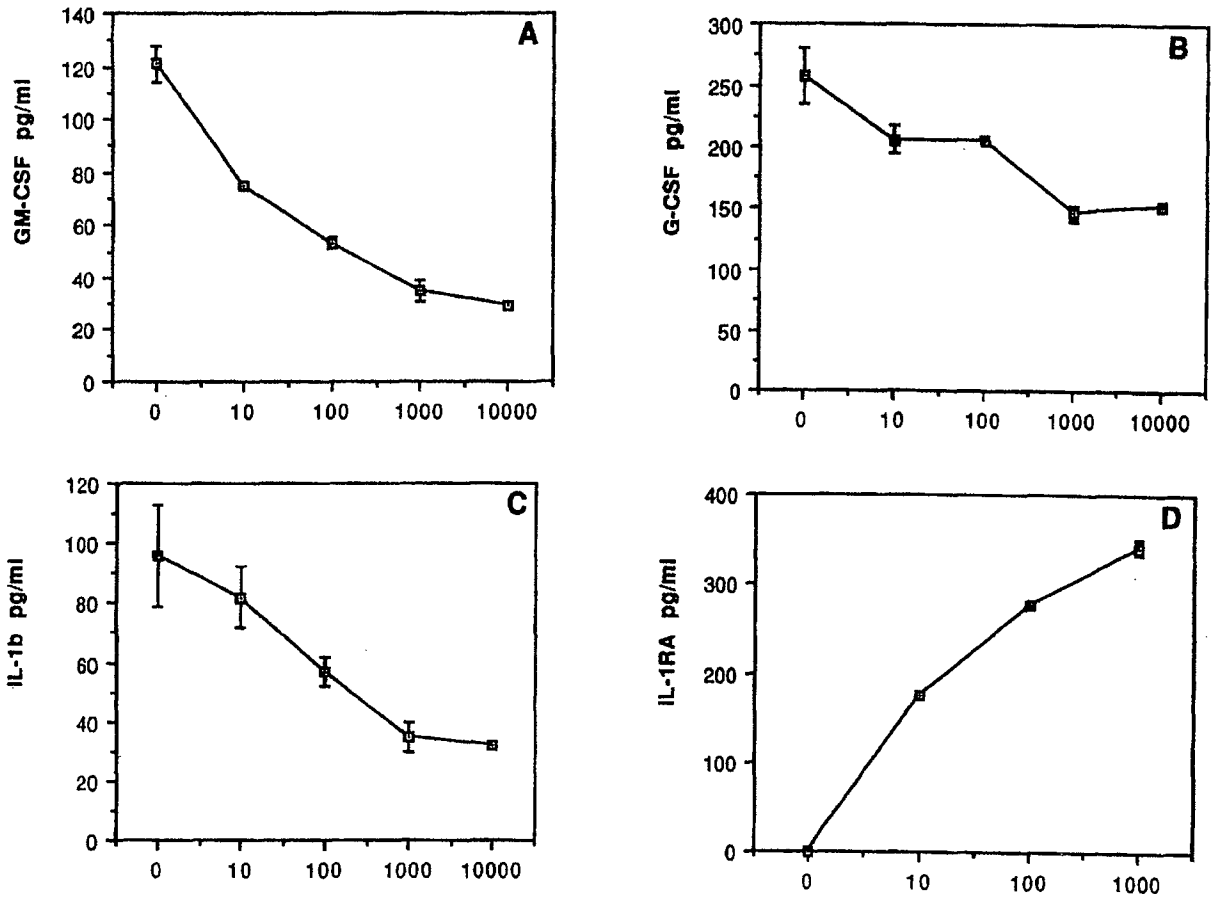

Fig 4. Dose dependence of the regulatory effects of IFN-α on the synthesis of cytokines. For measurement of GM-CSF, G-CSF, and IL-1β confluent secondary stromal cultures were incubated in medium containing 100 U/mL IL-1α with increasing concentrations of IFN-α (ordinate) for 24 hours. Concentrations of GM-CSF and G-CSF were measured in the supernatants, and IL-1β was measured in the lysates using specific ELISAs. IL-1RA was measured in supernatants under the same conditions without addition of IL-1α. Three experiments were carried out in triplicate and data from one representative experiment are presented as mean ± SEM.

GM-CSF and IL-1β at different quantitative levels. The strongest signals were observed on stimulation with IL-1α, followed by LPS and TNF-α. Expression of IL-1RA mRNA was only weakly induced by LPS and IL-1α, and observed only occasionally in the presence of TNF.

The influence of IFN-α on the expression of these cytokines was studied in exogenously stimulated stromal cell cultures. In stromal cells stimulated with IL-1α, LPS, or TNF, the expression of GM-CSF and IL-1β was markedly inhibited at a concentration of IFN-α 1,000 U/mL (Fig 1). In contrast to the cytokines with stimulatory effects on hematopoiesis, IL-1RA mRNA was induced by IFN-α at a much higher level as observed with LPS, suggesting a potent suppression of IL-1 mediated biologic actions. G-CSF expression, which was induced by IL-1α and TNF, was also downregulated by IFN-α. However, the inhibitory effect was less pronounced as compared with GM-CSF and IL-1β (data not shown).

Effect of IFN-α on cytokine secretion by bone marrow stromal cells. Protein levels of GM-CSF, G-CSF, IL-1β, and IL-1RA were evaluated in stromal cell supernatants using specific ELISAs. Unstimulated stromal cells failed to produce detectable levels of GM-CSF and G-CSF, and very low levels of IL-1RA were observed in only two of five experiments. In the presence of IL-1α or LPS, significant amounts of GM-CSF or G-CSF were detected, whereas TNF-α failed to induce G-CSF secretion and stimulated only very low levels of GM-CSF. IL-1β was never detected in stromal cell supernatants, independent of the stimulant added. The effect of IFN-α on the secretion of colony-stimulating factors was evaluated in cultures, which were stimulated with IL-1α (Fig 2). Results obtained from five individual experiments showed a significant reduction of GM-CSF secretion by IFN-α (1,000 U/mL) from 96.09 ± 10.38 pg/mL to 28.2 ± 6.91 pg/mL (mean ± SEM), representing a reduction of 72.42% ± 10.38%. The reduction of G-CSF secretion by IFN-α was less pronounced and amounted to 40% (Fig 2B).

Because IL-1β was not detected in stromal cell supernatants, despite readily detectable mRNA expression, we evaluated cell-associated IL-1β in stromal cell lysates. In fact, in cell lysates prepared by sonification of stromal cells, IL-1β production was seen on stimulation with either TNF, IL-

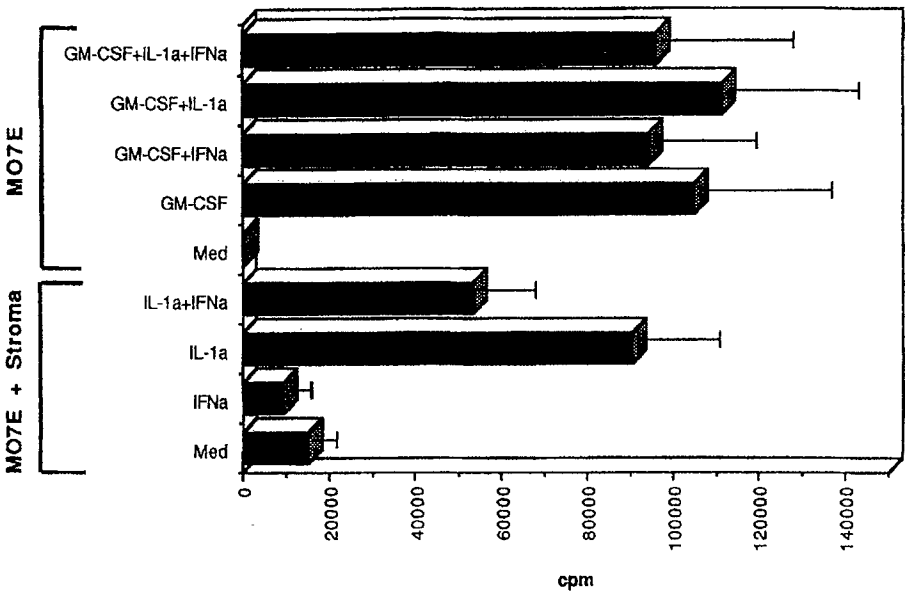

Fig 5. Inhibition of stroma-dependent proliferation of MO7e cells by IFN-α. MO7e-cells (1.0×10^4) were incubated in 96-well plates with or without preformed stromal layers with the indicated factors, and a proliferation assay was carried out as described in Materials and Methods. Six experiments were carried out in triplicate and data from all experiments are summarized as mean ± SEM. Concentrations used: IFN-α 1,000 U/ml, IL-1α 100 U/ml, GM-CSF 50 U/ml.

1α, or LPS (Fig 3A). In such induced cultures, cell-associated IL-1β was induced at comparable levels. Addition of IFN-α drastically reduced cell-associated IL-1β independent of the stimulus used, as shown in Fig 3A in one representative experiment. In four independent experiments, the mean reduction of IL-1β production by IFN-α in IL-1α stimulated stromal cells was 68% ± 14% (mean ± SEM). The strong induction of IL-1RA by IFN-α, as indicated by the mRNA analysis, was confirmed at the protein level (Fig 3B). IFN-α caused a more than 30-fold increase of IL-1RA secretion as compared with unstimulated cultures. In the presence of LPS, IL-1α, or TNF no additive effect was observed in the combination with IFN-α (data not shown).

The dose dependence of the modulatory effects of cytokine secretion by IFN-α was evaluated at IFN concentrations between 10 and 10,000 U/mL (Fig 4). Apparently, GM-CSF (Fig 4A) and IL-1RA (Fig 4D) secretion was most sensitive to the IFN-α action. However, significant effects of IFN-α were seen at concentrations of 10 to 100 U/mL for all cytokines evaluated in this study.

Biologic significance of IFN-α-mediated downregulation of GM-CSF. The consequences of the downregulation of hematopoietic growth factors by IFN-α were evaluated using the GM-CSF dependent cell line, MO7e. As shown in Fig 5, GM-CSF-induced proliferation of MO7e cells cannot be inhibited by IFN-α. Furthermore, IFN-α failed to induce expression of the Mx gene, confirming the IFN resistance of this cell line (data not shown). IL-1α had no direct proliferative effects on MO7e cells, neither alone, nor in combination with GM-CSF (Fig 5 and data not shown). When MO7e cells were incubated in confluent stromal cultures, a moderate proliferation was observed without stimulation with an additional growth factor, which was presumably mediated by stromal cell derived growth factors. The addition of IL-1α to the stromal cultures, resulting in the induction of GM-CSF, caused an increase of thymidine incorporation in MO7e cells, which was comparable to that induced by GM-CSF. IFN-α partially inhibited the indirect stimulation of MO7e cells by IL-1α in stromal cell cultures (Fig 5). To prove that GM-CSF represents the stimulatory factor for MO7e cells produced by stromal cells, a neutralizing antibody against GM-CSF was used in conditioned media from stromal cultures. MO7e cells were incubated with stromal cell supernatants stimulated and, as a control, with GM-CSF or IL-3.

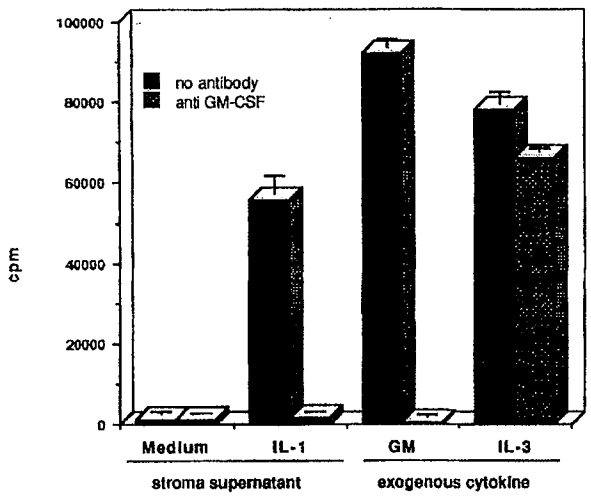

Fig 6. Stimulation of MO7e cells by stroma supernatants is inhibited by anti-GM-CSF antibody: Stromal cells were incubated for 48 hours in medium with and without IL-1α. The supernatants were harvested and tested for MO7e-stimulating activity in a 1:2 dilution with and without 10 μg/mL monoclonal anti-huGM-CSF antibody. In control wells MO7e cells were incubated with exogenous GM-CSF (10 U/mL) or IL-3 (5 ng/mL) with and without anti-GM-CSF. Three experiments were carried out (each in triplicate) and data are presented as mean ± SEM.

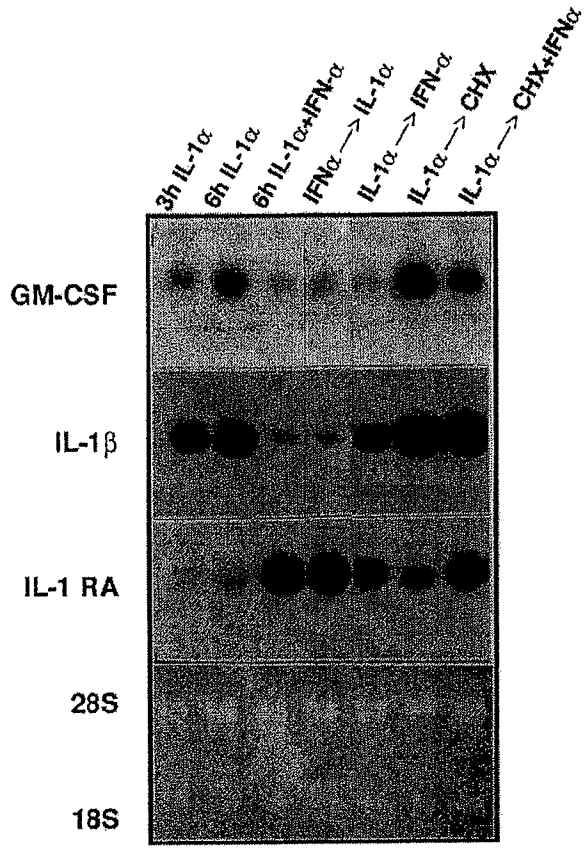

Fig 7. Role of de novo protein synthesis on the regulation cytokine expression by IFN-α. Stromal cells were incubated with IL-1α with or without IFN-α (lanes 1 to 3), or were pretreated with the first factor as indicated for 3 hours and supplemented with the second factor for a further 3 hours (indicated by arrows). Subsequently, cells were lysed and subjected to Northern-blot analysis. Concentrations used: IFN-α 1,000 U/mL, IL-1α 100 U/mL, CHX 10 μg/mL.

Anti–GM-CSF antibody completely inhibited GM-CSF-induced but not IL-3–induced proliferation of MO7e cells (Fig 6). IL-1α-induced stroma supernatants induced proliferation comparable to GM-CSF, which was completely blocked by the antibody, clearly suggesting that GM-CSF is the stimulatory cytokine in the conditioned medium. Similar results were obtained when MO7e cells were cocultured on IL-1α stimulated stromal layers and anti–GM-CSF antibody was added (data not shown). Based on the resistance of MO7e cells to a direct antiproliferative effect of IFN-α, it can be assumed that in stromal cell cultures the inhibition of MO7e cells by IFN-α is mediated by the downregulation of stimulatory cytokines, such as GM-CSF.

Requirement of protein synthesis for IFN-α action. In experiments using CHX as an inhibitor of protein synthesis, a different pattern of gene expression was observed for IL-1β and GM-CSF in response to IFN-α. When CHX was added simultaneously with IL-1α, upregulation of both cytokines was prevented, suggesting that de novo protein synthesis was required for IL-1α-mediated cytokine induction (data not shown). In IL-1α-stimulated cultures, the addition of IFN-α after three hours still resulted in a strong inhibition of GM-CSF expression, as was the case when IFN-α was added first, followed by IL-1α (Fig 7). Therefore, IL-1α was added first, followed by CHX with or without IFN-α added 3 hours later. As shown in Fig 7, CHX caused a superinduction of GM-CSF mRNA expression, which was clearly downregulated by IFN-α. For the upregulation of IL-1RA by IFN-α, protein synthesis was not required. By contrast, IFN effects on IL-1β expression appeared to be mediated by a different mechanism. In comparison to cultures receiving IL-1α and IFN-α simultaneously or IL-1α after IFN-α, the downregulation of IL-1β expression was less pronounced when IL-1α was added first, followed by IFN-α 3 hours later. In the presence of CHX, IFN-α failed to inhibit IL-1β expression suggesting that protein neosynthesis was necessary for this inhibitory effect of IFN-α.

Influence of actinomycin D on downregulation of cytokine gene expression. For the RNA decay analysis, foreskin fibroblasts were used, which responded to IFN-α by downregulation of GM-CSF and IL-1β expression, as observed in bone marrow stromal cells (data not shown). When the RNA synthesis was inhibited by actinomycin D, the addition of IFN-α did not result in an enhanced decay of GM-CSF or IL-1β mRNA, suggesting that a posttranscriptional mechanism was not involved in the inhibitory effect of IFN-α on the expression of these cytokines (Fig 8).

DISCUSSION

Suppression of hematopoiesis is a well-established feature of IFN-α, representing the basis for the therapeutic application in myeloproliferative diseases.[19-21] In vitro IFN-α inhibits proliferation of committed hematopoietic progenitor cells,[22] which is considered to be the explanation for its myelosuppressive action in vivo. We now demonstrate that IFN-α potently inhibits the paracrine expression of hematopoietic growth factors in bone marrow stromal cells. The potency of IFN-α as a regulator of cytokine cascades has only recently been recognized. The induction of IL-1RA in PBMNC by IFN-α[24] and the inhibitory effect of type I IFNs on the expression of IL-8 in a human fibroblast line,[29] bone marrow stromal cells, and hematopoietic cells[23] suggested an antiinflammatory role of IFN-α. Our present results, demonstrating antagonization of IL-1 action and inhibition of GM-CSF expression by IFN-α in bone marrow stroma, are in keeping with this biologic function. However, indirect suppression of hematopoiesis appears to be the most striking functional consequence of this novel action of IFN-α.

Hematopoietic cytokines are important mediators of stromal cell-supported hematopoiesis in vitro and probably also in vivo. Although GM-CSF is rarely detected in the supernatant of unstimulated Dexter stroma, in several reports constitutive production of biologically active GM-CSF is described within the adherent layer,[12,13] which is bound to glycosaminoglycans.[30] Thus, inhibition of stromal cell-dependent hematopoiesis appears to be a logical consequence of IFN-induced downregulation of colony stimulating factors.

IL-1 and IL-6 directly act on immature progenitor cells by a synergistic activity with colony-stimulating factors,[31] formerly described as hemopoietin-1 effect.[32,33] In addition,

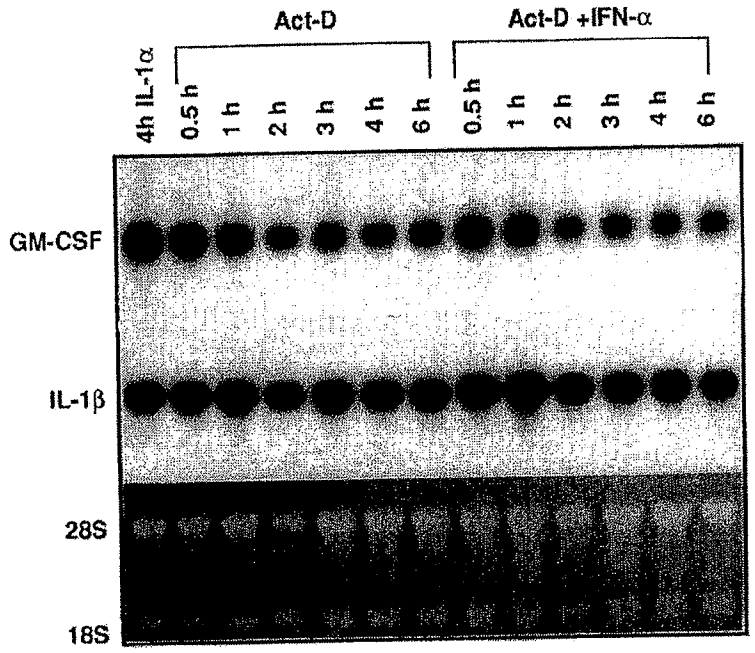

Fig 8. RNA decay analysis of GM-CSF and IL-1β. Subconfluent foreskin fibroblasts were incubated with IL-1α (100 U/mL) for 4 hours to achieve maximal induction of both cytokines. Subsequently, parallel cultures were incubated with actinomycin D (10 μg/mL) alone or with IFN-α (1,000 U/mL) for the indicated times. RNA was prepared and subjected to a Northern blot analysis. Two experiments were carried out and data of one experiment are presented.

IL-1 is a potent stimulant of GM-CSF and IL-6 production, leading to enhanced hematopoiesis in human long-term cultures.[10] Therefore, the inhibition of IL-1β and the concomitant induction of IL-1RA in bone marrow stromal cells by IFN-α are expected to counteract both the direct and the GM-CSF-mediated hematopoietic activity of IL-1. Although IL-1β mRNA was readily detectable in induced stromal cells, we failed to detect IL-1β protein in stromal cell supernatants. Most studies on cytokine production in bone marrow stromal cells examined mRNA expression only[11] or used concentrated supernatants[34] to detect IL-1 protein. After lysis of stromal cells by sonification, significant levels of immunoreactive IL-1β were measured in the present study, suggesting that cell-associated IL-1β is the predominant species of this protein in bone marrow stroma.

Suppression of IL-1β production, which was induced by IL-1α in monocytes, has recently been described for IFN-γ and IFN-α. However, in monocytic cells, IFN-α failed to suppress LPS-mediated IL-1 expression.[35] Interestingly, in bone marrow stromal cells, IFN-α significantly inhibited both cytokine- and endotoxin-stimulated IL-1β production, suggesting that the mechanism of IFN-α to regulate cytokine cascades might be different in hematopoietic and stromal cells.

The biologic significance of IFN-α mediated suppression of hematopoietic growth factors was evaluated using the factor dependent cell line, MO7e. In conventional long-term cultures IFN-α inhibits the proliferation of hematopoietic progenitors.[36] However, it would be impossible to distinguish whether IFN-α suppresses long-term hematopoiesis predominantly by direct antiproliferative effects or by indirect antihematopoietic effects, due to the downregulation of hematopoietic growth factors. Therefore, a factor-dependent cell line, which is resistant to direct action of IFN-α, as shown for the MO7e cell line, represents an ideal target to clarify this issue. The inhibition of stromal cell-supported proliferation of MO7e cells by IFN-α strongly suggests that downregulation of GM-CSF in stromal cells results in the functional suppression of hematopoiesis.

Downregulation of IL-1β and GM-CSF mRNA expression by IFN-α appears to be regulated at a transcriptional level. A similar mechanism has been postulated for the inhibition of IL-8 expression by IFN-α in a human fibroblast line.[29] However, experiments using CHX as an inhibitor of protein synthesis showed that inhibition of IL-1β expression seems to require the synthesis of an IFN-α-induced protein. The nature of IFN-α-induced intermediate proteins, which are operative in IL-1 inhibition, remains to be determined. By contrast, regulation of GM-CSF and IL-1RA expression is not affected by the presence of CHX, suggesting a direct action of IFN-α.

The biologic importance of this novel action by IFN-α for the regulation of hematopoiesis in vivo is unclear. It can be assumed, however, that myelosuppressive effects of IFN-α, as observed in therapeutic administration or during viral infection, might be at least partially based on the inhibition of the paracrine production of hematopoietic growth factors. Downregulation of hematopoietic cytokines might contribute to the beneficial effect of IFN-α in CML. The reduction of the paracrine secretion of hematopoietic growth factors, which appears to be enhanced by a constitutive overexpression of IL-1 in some patients,[37] might result in an overbalance of negative regulatory cytokines in the bone marrow microenvironment. Thus, in combination with other biologic actions, the influence of IFN-α on cytokine synthesis might contribute to the suppression of the malignant clone by IFN-α in the early phase of the disease. Significant inhibition of GM-CSF and IL-1β production, as well as induction of IL-

1RA, are observed in vitro at lower concentrations of IFN-α than required for direct antiproliferative action on hematopoietic progenitors. Therefore, endogenous IFN levels, induced by viral infections, might be sufficient for the down-regulation of growth factor production resulting in myeloid suppression. However, in context with bacterial infections, the strong induction of hematopoietic growth factors by bacterial products could overcome the suppressive effect of IFN, thus still allowing the expansion of the myeloid cell compartment.

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Exhibit C

United States Patent [19]

Cummins

[11] Patent Number: 4,497,795

[45] Date of Patent: Feb. 5, 1985

A3

[54] METHOD OF REGULATING APPETITE AND EFFICIENCY OF FOOD UTILIZATION EMPLOYING INTERFERON

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[58] Field of Search ..... 424/85; 260/112 R; 435/68, 811

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[57] ABSTRACT

The appetite of a warm-blooded vertebrate can be regulated by administering to the vertebrate a biological active fraction of interferon in an amount effective to modulate the vertebrate's food intake or efficiency in utilizing food. The appetite of a cow can be stimulated by the oral or intravenous administration of bovine fibroblast interferon or by interferon secreted nasally by the cow in response to inoculation with a vaccinal virus strain such as that of infectious bovine rhinotracheitis virus. The appetite of swine can be enhanced by oral administration of bovine fibroblast interferon.

18 Claims, No Drawings

METHOD OF REGULATING APPETITE AND EFFICIENCY OF FOOD UTILIZATION EMPLOYING INTERFERON

BACKGROUND OF THE INVENTION

This invention relates generally to a novel method for regulating the appetite of warm-blooded vertebrates. More particularly, this invention concerns the use of interferon isolates to modulate the appetite and increase the efficiency of food utilization for animals such as cattle, swine, and chickens.

"Interferon" is a term generically comprehending a group of vertebrate glycoproteins and proteins which are known to have various biological activities, such as antiviral, antiproliferative, and immunomodulatory activity in the species of animal from which such substances are derived. The following definition for interferon has been accepted by an international committee assembled to devise a system for the orderly nomenclature of interferons: "To qualify as an interferon a factor must be a protein which exerts virus nonspecific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein." *Journal of Interferon Research,* 1, pp. vi (1980).

Since the first descriptions of interferon by Isaacs and Lindeman [See, Proc. Roy. Soc. London (Ser.B), Vol. 147, pp. 258 et seq. (1957) and U.S. Pat. No. 3,699,222], interferon has been the subject of intensive research on a worldwide basis. Publications abound concerning the synthesis of interferon, its proposed molecular characterizations, its clinical applications, and proposed mechanisms of its antitumor, antiviral, and immune system activities. See, for example, DeMaeyer, et al., "Interferons" appearing as Chapter 5 in *Comparative Virology,* Vol. 15, pp. 205–284, Plenum Press, N.Y., N.Y. (1979); Cantrell, "Why Is Interferon Not In Clinical Use Today" appearing in *Interferon* 1979, I. Gresser, ed., Vol. 1, pp. 1–28, Academic Press, London (1979); Stewart, "The Interferon System" Springer-Verlag, N.Y., N.Y. (1979); and Dunnick, et al., "Clinical Trials with Exogenous Interferon", *J. Infect. Diseases,* 139, No. 1, pp. 109–123 (1979).

Because of the intensity and disparate origins of research concerning interferon and its characteristics and uses, there exists a substantial lack of uniformity in such matters as classification of interferon types. There are also numerous, sometimes contradictory, theories concerning the mode of action of interferon in producing clinical effects. The following brief summary of the current state of knowledge regarding interferon will aid in understanding the present invention.

Although originally isolated from cells of avian origin (chick allantoic cells), interferon production has been observed in cells of all classes of vertebrates, including mammals, amphibians, and reptiles. Interferon production by vertebrate cells is seldom spontaneous but is often readily "induced" by treatment of cells (in vivo or in vitro) with a variety of substances including viruses, nucleic acids (including those of viral origin as well as synthetic polynucleotides), lipopolysaccharides, and various antigens and mitogens.

Interferon have generally been named in terms of the species of animal cells producing the substance (e.g., human, murine, or bovine), the type of cell involved (e.g., leukocyte, lymphoblastoid, fibroblast) and, occassionally, the type of inducing material responsible for interferon production (e.g., virus, immune). Interferon has been loosely classified by some researchers according to induction mode as either Type I or Type II, with the former classification comprehending viral and nucleic acid induced interferon and the latter class including the material produced as a lymphokine through induction by antigens and mitogens. More recently, the international committee devising an orderly nomenclature system for intereferon has classified interferon into types on the basis of antigenic specificities. In this newer classification, the designations alpha (α), beta (β), and gamma (γ) have been used to correspond to previous designations of leukocyts, fibroblast, and type II (immune) interferons, respectively. Alpha and beta interferons are usually acid-stable and correspond to what have been called type I interferons; gamma intereferons are usually acid-labile and correspond to what has been called type II intereferons. The international committee's nomenclature recommendations apply only to human and murine interferons. *Journal of Interferon Research,* 1, pp. vi (1980). Therefore, the interferon employed herein is identified simply by animal species and type of cell producing the intereferon, e.g. bovine fibroblast interferon.

Determination of precise molecular structures for interferon was for some time beyond the capacities of the art. In the years since interferon was first characterized as proteinaceous on grounds of its inactivation by trypsin, attempts to purify and uniquely characterize it have been frustrated by its high specific activity as well as its apparent heterogeneity. Presently, some precision in determining molecular structure has been achieved for interferon derived from a single cell type and using a single specific inducer, e.g., human alpha interferon.

In its earliest applications, interferon was employed exclusively as an antiviral agent and the most successful clinical therapeutic applications to date have been in the treatment of viral or virus-related disease states. It became apparent, however, that exogenous interferon was sometimes capable of effecting regression or remission of various metastatic diseases. A summary of clinical trials of interferon as an antiviral and antiproliferative therapeutic agent through late 1978 is contained in Dunnick, et al. supra.

The clinical agent of choice in this work has been human leukocyte interferon, "mass-produced" by procedures involving collection and purification of vast quantities of human buffy cost leukocytes, induction with virus, and isolation from culture media. The need for interferon of human source is, of course, consistent with the long-standing conclusion that interferon is "species specific", i.e., biologically active, in vivo, only in species homologous to the source cells.

In the work described above, interferon has been administered parenterally, i.e., intramuscularly and intradermally, with some successful topical usages having been reported. It has seldom been administered intravenously because of substantial adverse effects attributable to "contaminants" in crude and even highly purified isolates. Prior to applicant's invention described in U.S. patent application, Ser. No. 180,464, filed Aug. 22, 1980, and in PCT International Application No. PCT/US 81/01103, filed Aug. 18, 1981, published Mar. 4, 1982, the disclosures of which are hereby incorporated by reference, there had been no reports of therapeutically successful oral administration of interferon. This circumstance was consistent with the widely held belief

that interferon would not withstand exposure to a digestive environment such as that found in mammals.

In addition to use in antiviral and antitumor therapy, interferon has rather recently been noted to possess immunomodulatory effects, both immunopotentiating and immunosuppressive in nature. See, e.g., Sonnenfeld, et al., "A Regulatory Role For Interferon In Immunity", Annals, N.Y. Acad. Sci., Vol. 322, pp. 345–355 (1979). While no human clinical or in vivo animal work specifically directed to evaluation of immunological effects of interferon has been reported, it is proposed by some that the antitumor effects of interferon are at least in part related to immune stimulation or activation of so-called "natural killer cells," macrophages and T-lymphocytes. See, e.g., Kershner, "New Directions in Cancer Chemotherapty" *A.S.M.News,* Vol. 46, No. 3, pp. 102 et seq. (1980).

Further, "new" biological activities for exogenous interferon are consistently being ascertained. Cantrell, et al., *New Eng. Jour. Med.,* Vol. 302, No. 18, P. 1032 (1980) report an effect of interferon in transiently diminishing high density lipoprotein levels and total cholesterol values, suggesting that interferon in humans, may influence cardiovascular disease.

Prior to applicant's invention described and claimed in the present application, there had been no reports of any biological activity of any form of interferon with a direct impact upon the appetite or efficiency of food utilization in vertebrates. Insofar as the possibility of using interferon to stimulate appetite is concerned, interferon has been considered in the art as possessing the opposite effect. It has been reported in the literature that human patients receiving interferon cancer therapy experience a loss of appetite as a side effect of such therapy. Marx, Science 210, p. 998 (1980); *Journal of Infectious Diseases,* 139, pp. 109–25 (1979). This suppression of appetite has been one of a number of side effects, such as lower white blood cell counts, nausea, fever, and hair loss, experienced by humans in clinical trials of interferon. Further, the prior art literature has not reported any effect of interferon upon appetite in nonhuman species.

SUMMARY OF THE INVENTION

According to the present invention, it has been discovered that the appetite of a warm-blooded vertebrate can be regulated by a method comprising administering to the warm-blooded vertebrate a biologically active fraction of interferon in an amount effective to modulate the vertebrate's food intake or efficiency in utilizing food. The amounts of interferon effective to modulate food intake have been discovered to be much lower than those amounts of interferon necessary to realize its antiviral, antitumor, and modulatory effects. Though the exact mechanism by which interferon affects appetite remains unconfirmed, applicant believes that he has discovered an effect of interferon upon the satiety center and hunger centers of the brain and central nervous system, rather than a mere side effect or toxic effect realized from administration of interferon. This method of regulating appetite is presently believed to be of the most value in stimulating the appetite of mammals but also finds application in modulation of the food intake or efficiency of food utilization of any warm-blooded vertebrates, including avian species.

Interferon derived from any cell source may be used in the method of the present invention. Genetically engineered interferon may also be used. Fibroblast interferon found in cells of bovine species origin is the presently preferred type of interferon, principally because of its easy availability in relatively large quantities.

In the method for stimulating the appetite of cattle which also forms part of the present invention, cattle are orally administered a biologically active fraction of fibroblast interferon derived from cells of bovine species origin. When orally administered, cattle should receive at least one dose of at least 10,000 units of such interferon per kg of body weight.

Though oral administration of the bovine fibroblast interferon is preferred, the appetite of cattle may also be stimulated by intravenous administration of the bovine fibroblast interferon. When intravenously administered, each bovine should be given daily for at least three days at least one dose of about 4,000 units of such interferon per kg of body weight.

Though bovine fibroblast interferon is preferred for use in stimulating the appetite of cattle, the interferon may also be secreted nasally by the cattle in response to innoculation with a vaccinal virus strain, such as that of infectious bovine rhinotracheitis (IBR) virus. Each bovine may be innoculated with at least about 10^4 $TCID_{50}$ of such vaccinal strain.

Also forming a part of the present invention is a method for stimulating the appetite of swine comprising administration of a biologically active fraction of interferon. At present, this method of stimulating the appetite of swine entails the oral administration of bovine fibroblast interferon to piglets prior to weaning. Preferably, each pig is given from about 5,000 to about 50,000 units per kg of body weight of such interferon per day for one to five days before weaning.

A method for increasing the efficiency of food utilization in chickens also forms a part of the present invention. This method comprises administering a biologically active fraction of interferon glycoprotein, the preferred interferon being bovine fibroblast interferon. Preferably, the chickens receive the bovine fibroblast interferon in their drinking water, in an amount at least about 70 units per ml of drinking water.

Examples of the more important features of this invention have thus been summarized rather broadly in order that the detailed description thereof that follows may be better understood, and in order that the contribution to the art may be better appreciated. There are, of course, additional features of the invention that will be described hereinafter and which will also form the subject of the claims appended thereto.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As employed throughout this application, the term "interferon" shall have the meaning ordinarily attributed thereto in the art, including but not limited to the meaning ascribed thereto in U.S. Pat. No. 3,699,222.

Interferon of human and murine origins has been quantified in the art in terms of International Units ("IU"), notwithstanding knowledge that, for example, the molecular weight of human leukocyte and lymphoblastoid ranges between 13,000 and 25,000 daltons. As used herein, a "unit" of interferon shall mean the reciprocal of a dilution of interferon-containing material that, as determined by assay, inhibits one-half of a challenge virus plaque, the challenge virus being the vesicular stomatitis virus (VSV).

Unless otherwise indicated, as used throughout the examples presented herein, "bovine fibroblast interferon, "bovine IFN," or "IFN" shall means that interferon which has been prepared in accord with the procedures of Example 1.

EXAMPLE 1

Primary bovine fetal kidney (BFK) or bovine testicular (BT) cells were grown to confluency in cell culture. Stocks of bluetongue virus (international serotype 10) were prepared in baby hamster kidney (BHK) cells or VERO cells and had titers of 10^6 to 10^8 plaque forming units (PFU)/ml. The BFK or BT cells were challenged with bluetongue virus (multiplicity of infection of greater than 1 was best), and supernatant fluids were generally harvested when the cytopathic effect (CPE) involved the entire cell sheet, i.e., about 24 to 48 hours. The supernatant fluids were dialyzed for 24 hours in a KCl-HCl buffer (ph 2.0) and for 24 hours in a phosphate buffered saline (ph 7.4) before ultracentrifugation at 100,000×g for 60 minutes. The interferon activity (expressed as "units" as opposed to IU) was assayed by a plaque reduction method using VSV as a challenge virus on BFK cells Rosenquist and Loan, "Interferon Production With Strain SF-4 of Parainfluenza-3 Virus" *Am. J. Vet. Res.*, 28, pp. 619–628 (1967).

While phosphate buffered saline was used as a carrier for interferon, other pharmaceutically acceptable diluents, adjuvants, and carriers of the type commonly used in oral and parenteral therapy must be employed.

EXAMPLE 2

A set of healthy guinea pigs, already consuming near maximum amounts of food, were used to test the bovine fibroblast interferon of Example 1 for toxicity. Each animal was orally administered the amount of bovine IFN indicated in Table A. The particular IFN preparation given in this study had a titer of 3000 units/ml. As indicated in Table A, those guinea pigs given IFN gained an average of 30 grams each, while the controls lost an average of 20 grams.

TABLE A

Guinea Pigs Administered Oral IFN or Placebo for Seven Days

Animal No:	Treatment Group	Starting Weight (gms)	Finishing* Weight (gms)	Net Change (gms)
1	Control	475.4	457.4	−18.0
2	Control	429.5	407.2	−22.3
		904.9	864.6	−40.3
3	IFN Once Daily (2 ml/day)	450.0	493.8	+43.8
4	IFN Once Daily (2 ml/day)	378.6	432.8	+54.2
		828.6	926.6	+98.0
5	IFN Twice Daily (4 ml/day)	428.3	419.7	−8.6
6	IFM Twice Daily (4 ml/day)	432.7	465.0	+32.3
		861.0	884.7	+23.7
Total	All Animals	2,594.5	2,675.9	+81.4
Average	All Animals	432.425	445.983	+13.558
Average	IFM Only	422.400	452.825	+30.425
Average	Controls Only	452.450	432.300	−20.150

*Finishing weight taken 10 days after starting weight.

EXAMPLE 3

In another trail involving guinea pigs, ten pigs were used as controls against eight pigs that were orally administered 1 cc/day of a bovine IFN preparation having a titer of 8000 units/ml. The data from this study are summarized in Table B. Bovine IFN was administered for five days only, and all guinea pigs were observed for ten days more. During the first five days of treatment, each group was consuming nearly the same amounts of food, but the IFN-treated guinea pigs ate 8.1% more after treatment, and the controls ate 1.2% less.

TABLE B

Daily Food Consumption (Grams) per Guinea Pig per Group - IFN Administered vs. Controls

Period	Group (No.) IFN (8)	Controls (10)
1st 5 days	38.2	38.4
2nd 5 days	40.0	37.6
3rd 5 days	41.3	38.0
Change (%):		
1st 5-2nd 5	+4.7	−2.2
1st 5-3rd 5	+8.1	−1.2

EXAMPLE 4

Male broiler chickens were tested for two weeks to provide data on the effect of bovine IFN on efficiency of feed utilization and feed consumption in chickens. This study involved four groups of sixteen baby chicks each, one group being a control. The baby chicks were given bovine IFN in their drinking water at dilutions of 1:10, 1:100, and 1:1000, respectively, of a bovine IFN preparation having a titer of 7000 units/ml. As shown in Table C, the group of chicks receiving the 1:10 IFN dilution gave a favorable response. The groups of chicks all had nearly the same average finishing weight, but those treated with the 1:10 IFN dilution were 10.4% more efficient, requiring less food for a pound of weight gain.

TABLE C

Effect of Interferon on Efficiency of Feed Utilization and Feed Consumption in Male Broiler Chicks

Water Treatment (IFN units/ml)	Avg. wt. (gm)	Lb feed/ Lb gain	Feed consumed/ bird (gm)	H_2O consumed/ bird (mls)
0	194.0	1.54	299	456
700	194.5	1.38	268	496
70	194.8	1.60	312	436
7	192.3	1.53	293	439

EXAMPLE 5

In another study of the effect of bovine IFN on broiler chicks, forty male broiler chicks were tested, twenty treated with IFN and twenty acting as a control

group. The IFN-treated chicks had 700 units of IFN per ml added to their normal drinking water for two weeks. All chicks were observed and their weight gain monitored for eight weeks. As shown in Table D feed consumption was adversely affected at the particular IFN dosage under study.

The effect of IFN observed in examples 4 and 5 will be useful in preventing excessive weight gain in breeding flocks of the poultry industry. The appropriate dose of IFN will result in less feed consumption but increased efficiency in utilizing feed, resulting in less fat and more protein deposition.

TABLE D

Interferon Effect on Broiler Chicks When Supplied Via the Drinking Water

Treatment	Weights (gms)								Total ml H_2O	Feed Efficiency
Week	1	2	3	4	5	6	7	8	0–4 wks	0–8 wks
IFN-Treated	106.5	237.9	334.9	553.9	867.4	1106.8	1442.9	1533.5	1193.0	2.47
Control	105.8	243.4	358.1	569.4	915.6	1211.3	1537.2	1656.4	1221.7	2.30

EXAMPLE 6

A collection of calves, all suffering from severe illness and accompanying anorexia, were used to study the effects of orally administered bovine IFN. Ten of the calves acted as controls, and three of the calves were orally administered a single dose of 90 ml of a bovine IFN preparation with a titer of 7000 units/ml. As shown in Table E, the calves' total feed consumption for 4 days (Day 2-Day 5) prior to IFN administration was only 0.3–1.6 lbs., recorded by the pinpointer. A 400-lb calf normally eats 1% of its body weight the first week in the feedlot, and 2% the second week. Thus, all calves used in this study were exhibiting severe appetite suppression. Within two days of the single IFN treatment, the IFN-treated calves were all eating five pounds of feed or more, while only one of the ten controls was eating that much. All three IFN-treated calves survived the study, while only four of the ten controls survived.

TABLE E

Daily Consumption of Food by Sick Cattle

Calf No.	Days 2–5*	6**	7	8	9	10	11	12	Fate 2 Months After Test Commenced
Controls									
63	0.3	0	0	0			Dead		Died
77	1.6	0	0	0	0	0	Pulled		Survived
85	1.2	0	0	0	0	0	Pulled		Died
91	0.8	0	0	0	0	0	Dead		Died
143	0.9	0.3	2.2	5.6	9.8	8.3	9.2	8.7	Survived
191	0.3	0	0	0	0	2.3	2.7	Pulled	Survived
254	0.7	0	0.6	0	0	0	Pulled	Dead	Died
290	0.3	0	0	0			Dead		Died
299	0.7	0	0	0	1.3	4.5	5.6	8.6	Survived
375	0.4	0	0	0	0	0.7	Pulled	Dead	Died
IFN Treated									
44	0.8	0.4	1.1	5.0	10.2	5.0	5.6	10.9	Survived
112	0.3	0	4.1	10.2	9.6	14.7	16.1	15.4	Survived
173	1.2	0.4	2.4	5.3	6.2	2.5	1.3	2.8	Survived

Numbers under days represents pounds of food consumed that day.
"Pulled" means the calf was treated for illness and removed from the pinpointer.
*Total pounds of food consumed on days 2–5.
**IFN administered on the morning of the 6th day.

EXAMPLE 7

A test was conducted to study the effect of bovine IFN on Pasteurella-vaccinated calves. Thirty-seven (37) calves were intradermally vaccinated with 0.5 ml of A. H. Robins' Pasteurella vaccine (live Pasteurella hemolytica, serotype 1), while 45 were given a placebo injection. At least 20 days after the vaccinations, 24 of the unvaccinated calves and 18 of the vaccinated were each administered a single oral dose of bovine IFN with a titer of 7000 units/ml. The remaining calves were each given a placebo. Each IFN-treated calf received 120 ml of the IFN if it weighed more than 400 lbs or 90 ml of the IFN if it weighed less than 400 lbs. The food consumption and weight gain averaged for each group at seven days after treatment with IFN are reported in Table F. Vaccinated calves given IFN ate 40% more the first week thereafter than vaccinated calves given a placebo. Unvaccinated calves given a placebo ate 13% more the first week thereafter than unvaccinated calves given IFN. The different response between vaccinates and controls may have been due to some interaction between IFN treatment and vaccination that may have produced more severe appetite suppression in vaccinates. Also, the comparison between IFN-treated and control calves had more validity for the vaccinated calves because the average weights of the IFN-treated and control calves were much closer than for the unvaccinated calves. In the unvaccinated group, the controls were heavier than the IFN-treated calves, and thus probably older and more likely to perform better.

TABLE F

Effect of Oral IFN Treated on Pasteurella-Vaccinated Calves - 7-Day Data on 82 Calves Consuming Food as Determined by Pinpointer

Group	No. of Calves	Average Weight	Group Weight	7-Day Consumption	Avg. Daily Consumption	% of Avg. Wt. Consumed Daily
Unvaccinated No IFN	24	420.2	10,086	1,041.6	6.2	1.50
Unvaccinated IFN	21	391.8	8,228	761.1	5.2	1.33
Vaccinated IFN	18	412.4	7,424	832.8	6.6	1.63
Vaccinated No IFN	19	407.1	7,734	623.3	4.7	1.16

EXAMPLE 8

Two calves that were consuming an average of 14.6 lbs of feed per day for four days were intravenously given 800,000 units of bovine IFN on each of four days (Days 0, 1, 2, and 3 of Table G). On each day of the IFN treatment, each calf consumed less than 14.5 lbs, even dropping as low as 6.5 and 6.7 lbs respectively on the last day of treatment. After completion of the IFN treatment itself, feed consumption for both calves increased substantially.

TABLE G

Feed Consumption (lbs) by Calves Given Bovine IFN Intravenously

Calf No.	Day After Intravenous Inoculation 0	1	2	3	4	5	6	7	8	9	10	11
461	13.0	11.1	13.8	6.5	17.4	23.3	22.4	17.7	23.4	19.6	21.9	27.8
490	13.4	12.0	12.3	6.7	16.4	14.0	21.5	17.5	15.4	17.6	20.9	18.7

EXAMPLE 9

In this study, the source of interferon was nasal secretions induced by vaccination with a vaccine (TSV-2) prepared from temperature sensitive mutant of infectious bovine rhinotracheitis (IBR) virus and parainfluenza-3 (PI-3) virus. The temperature sensitive strain of attenuated IBR virus was prepared by Norden Laboratories of Lincoln, Nebr. Materials and methods used in preparing the temperature sensitive strain of IBR virus are described in more detail in "Evaluation of the Safety and Efficacy of an Intranasal Vaccine Containing a Temperature-Sensitive Strain of Infectious Bovine Rhinotracheitis Virus", *Kucera* et al, Am J Vet Res, 39, 607–10 (1978). The TSV-2 vaccine also contains a temperature sensitive strain of PI-3. Temperature sensitivity means that the viruses are treated so that they cannot replicate at the body temperature of the cow and their growth is restricted to the nasal mucosa. Eight calves were given TSV-2, and eight calves were treated as controls. One ml of the TSV-2 vaccine containing about 300,000 $TCID_{50}$ of IBR virus was administered to each nostril of each treated calf. The complete virus titers for the TSV-2 vaccine administered in the amount recommended by Norden Laboratories is shown in Table H. After vaccination, each calf was monitored to record weight gain and its daily feed consumption determined by pinpointer. Table I reports the feed consumption data for each calf and shows the vaccinated calves consumed more food than the unvaccinated controls. Table J reports the body weight data and shows that the vaccinated calves gained more weight and did so more efficiently than the unvaccinated controls.

TABLE H

IBR/PI-3 Vaccine Dosages (Virus Liters)

Vaccine	Product Information (Virus Liters)
Anchor Labs (IM)	IBR $\geqq 10^{5.5}TCID_{50}$; PI-3 $\geqq 10^{5.8}TCID_{50}$
TSV-2	$IBR_{TS} = 10^{5.5}TCID_{50}$; $PI\text{-}3_{TS} = 10^{6.4}TCID_{50}$
Rhivin	IBR = $10^{6.1}TCID_{50}$; PI-3 = $10^{7.1}TCID_{50}$
Rhivin/10	IBR = $10^{5.1}TCID_{50}$; PI-3 = $10^{6.1}TCID_{50}$

TABLE I

Food Consumption (lbs) by Calves After Vaccination with Intranasally Administered IBR and PI-3 Vaccine (TSV-2)

Calf No.	Days After Vaccination 1–14	15–28	1–28
Controls:			
5	219	282	501
8	191	161	352
9	116	160	276
22	283	267	550
40	172	207	379
41	225	254	479
88	163	170	333
91	227	283	510
Total	1,596	1,784	3,380
Average	200	223	423
Daily Average	14.3	15.9	15.1
Vaccinates:			
1	273	273	546
10	272	415	687
12	114	154	268
50	180	146	326
79	199	230	429
82	132	189	321
85	221	294	515
93	219	305	524
Total	1,610	2,006	3,616
Average	201	251	452
Daily Average	14.4	17.9	16.1

TABLE J

Body Weights of Calves Following Vaccination with Intranasally Administered IB and PI-3 Vaccine (TSV-2)

Calf No.	Days After Vaccination 0	14	28	Gain, 1–28
Controls:				

TABLE J-continued

Body Weights of Calves Following Vaccination with Intranasally Administered IB and PI-3 Vaccine (TSV-2)				
	Days After Vaccination			
Calf No.	0	14	28	Gain, 1-28
5	660	664	752	92
8	526	546	606	80
9	410	384	442	32
22	658	690	758	100
40	456	460	510	54
41	526	544	604	78
88	498	540	562	64
91	474	472	546	72
Total	4,208	4,300	4,780	572
Average	526	537.5	597.5	71.5
Average Daily Gain	—	0.82	4.29	2.55
Vaccinates:				
1	636	688	752	116
10	528	540	636	108
12	426	440	496	70
50	444	474	528	84
79	568	560	642	74
82	478	510	570	92
85	654	662	754	100
93	434	428	492	58
Total	4,168	4,302	4,870	702
Average	521	537.8	608.8	87.8
Average Daily Gain	—	1.20	5.07	3.13

The presence of interferon in the nasal secretions of each calf in the study was monitored for ten days after vaccination by intranasal administration of the TSV-2 vaccine. As shown in Table K, during the ten days after vaccination, IFN was detected in 56 of 76 (74%) and in 2 of 63 (3%) of nasal secretion samples collected from vaccinates and controls, respectively. From 3 through 8 days after vaccination, IFN was detected in 51 of 55 (93%) samples collected from vaccinates.

TABLE K

Interferon Titers in the Nasal Secretions of Calves After Intranasal Administration of IBR Vaccine (TSV-2)											
Calf	Day After Intravenous Inoculation										
No.	0	1	2	3	4	5	6	7	8	9	10
Controls:											
5	0	0	0	0	0	—*	0	—	0	0	0
8	0	0	0	0	0	—	0	0	0	0	0
9	0	—	0	0	0	—	—	50	0	—	0
22	0	—	—	0	0	0	0	0	0	0	—
40	0	0	0	0	0	0	0	—	0	0	0
41	0	—	0	0	0	—	0	0	0	0	0
88	0	—	42	0	0	0	0	0	0	0	0
91	0	0	0	0	—	0	—	—	0	0	0
**	0/8	0.4	1/7	0/8	0/7	0/4	0/6	1/5	0/8	0/7	0/7
Vaccinates:											
1	0	0	0	62	400	200	0	55	32	0	490
10	0	0	0	49	420	200	160	400	2000	33	0
12	0	23	42	—	46	200	0	33	32	100	320
50	0	62	0	20	500	230	160	49	110	0	0
79	0	0	—	80	110	150	370	270	1100	20	0
82	0	—	0	62	34	0	31	52	290	680	38
85	20	62	0	35	140	0	78	20	2000	700	440
93	0	—	0	250	450	120	200	44	44	0	0
**	1/8	3/6	1/7	7/7	8/8	6/8	6/8	8/8	8/8	5/8	4/8

*Quantity of nasal secretions insufficient for testing.
**Number of calves with interferon/number of calves tested.

All nasal secretion samples were dialyzed in a KCl-HCl buffer (ph 2.0) overnight, and then a PBS buffer (ph 7.4) overnight before assay by plaque reduction. The plaque reduction method, as modified by Rosenquist and Loan (Am J Vet Res 28:619-628, 1967), was used. Serial dilutions of the prepared sample were made in maintenance medium. Two ml amounts of these dilutions were applied to 6 well petri dish cultures of bovine fetal kidney cells, and allowed to remain overnight at 37° C. Control cultures were treated overnight with 2 ml of maintenance medium. After the incubation period, fluids were aspirated, plates were washed with 2 ml of Hanks' BSS, and 0.25 ml of VSV (calculated to contain 50 PFU) was added to each petri dish. After adsorption at 37° C. for 1 hour, excess viral fluids were aspirated, and the overlay medium was added. Plaques were usually scored on the third day. Interferon titers were determined by the probit method (Lindenman and Gifford, Virology 19:302-309, 1963) and were expressed as the reciprocals of the dilutions which produced 50% reduction in the number of VSV plaques, as compared with the number in control cultures.

EXAMPLE 10

The source of interferon in this study was nasal secretions induced by vaccination with a number of vaccines for infectious bovine rhinotracheitis (IBR). The feed consumption for all calves including 10 control calves used in this study was studied for four days prior to vaccination. Each calf in its vaccination group was allowed to eat from one of five pinpointers. Data on this observation of feed consumption in the calves is shown in Table L.

TABLE L

Food Consumed in Pounds, Total 4-Day Consumption Before Trial					
	Pinpointer Number				
Calf No.	1	2	4	6	8
1	40	64	66	45	72
2	60	60	56	54	67
3	59	41	50	46	26
4	48	56	49	44	00
5	14	54	42	NA*	35
6	42	46	61	00	45
7	66	74	71	32	55
8	36	60	06	24	47
9	32	BL**	64	42	34
10	43	00	38	71	31
Total	440	455	503	358	412

TABLE L-continued

Calf No.	Food Consumed in Pounds. Total 4-Day Consumption Before Trial — Pinpointer Number 1	2	4	6	8
Average	44.0	50.6	50.3	39.8	41.2
Avg. Daily Consumption	11.00	12.64	12.58	9.94	10.30

*Calf substituted on day 0 from pen.
**Bloating calf dropped from study.

In addition to the control calves, ten calves vaccinated with the intranasal IBR vaccine TSV-2 in the same manner as described in Example 9 were studied. This trial also included nine calves vaccinated with an intramuscular (IM) IBR/PI-3 vaccine manufactured by Anchor Labs, ten calves vaccinated with a full dose of an intranasal IBR/PI-3 vaccine (Rhivin) manufactured by Pitman-Moore, Inc., and ten calves vaccinated with one tenth of a full dose of the Rhivin vaccine. The dose rates for each of these vaccine treatments is shown in Table H.

For each group of calves in the study, the total gain and average daily gains during the 60-day pretrial period are shown in Table M. Data collected before the trial indicated some differences in their pretrial performance as shown in Tables L and M. Though the groups were not identical, the data do reflect that the treatment groups were balanced and provided fair comparisons.

TABLE M

Treatment	No. of Calves	Pretrial Data on Weights and Weight Gain: 60-Day Weight	0-Day Weight	Total Gain: Pretrial	Total Gain: Posttrial	Average Daily Gain: Pretrial	Average Daily Gain: Posttrial	Average Daily Gain: Change
Control	10	415.8	567.4	151.6	64.4	2.53	2.30	−0.23
Anchor Labs (IM)	9	420.2	564.1	143.9	69.0	2.40	2.46	+0.06
TSV-2	10	423.6	576.0	152.4	73.9	2.54	2.64	+0.10
RHIVIN	10	416.0	565.9	149.9	67.5	2.50	2.41	−0.09
RHIVIN/10	10	415.6	572.2	156.6	69.5	2.61	2.48	−0.13

Summary data for the trial following the specific treatments is presented in Table N. The calves treated with the TSV-2 intranasal vaccine demonstrated the greatest total gain, the best average daily gain, and the best efficiency of feed utilization.

TABLE N

Pen	Treatment	Summary Weights, Gains, Feed Consumption: Average Weight* Begin	Average Weight* End	Average Total Gain	Percent Increase	Average Daily Gain	Average Daily Feed Consumed	Feed/ Gain**
1	Control****	567.4	631.8	64.4	11.4	2.30	15.14	6.58
2	Anchor Labs (IM)***	564.1	633.1	69.0	12.2	2.46	15.41	6.26
4	TSV-2	576.0	649.9	73.9	12.8	2.64	15.89	6.02
6	RHIVIN	565.9	633.4	67.5	11.9	2.41	15.20	6.31
8	RHIVIN/10	572.2	641.7	69.5	12.1	2.48	16.34	6.59

*Beginning weight = average of 2 weights taken at 0 and 2 days. Ending weight = average of 2 weights taken at 28 and 29 days.
**Feed/gain calculated by dividing the average daily pounds of food consumed by the average daily gain.
***One calf removed from data of pen 2 because of bloating.
****Control calves seroconverted to IBR virus by 28 days indicating an inapparent IBR virus infection.

EXAMPLE 11

The effect of intranasal secretion interferon was tested in another study involving six bulls and six steers for each test group. One group was treated as a control, one group was vaccinated with the intranasal IBR vaccine TSV-2 in the same manner as in Example 9, and one group was vaccinated with the intramuscular IBR-/PI-3 vaccine in the same manner as in Example 10.

Table O shows the beginning average weight and the average weight gains per calf at 7, 14, 21, 28, and 58 days following vaccination. Table P presents the average daily weight gain and the average weight gain as a percent of body weight at 28 and 58 days. The vaccinated calves averaged greater daily weight gain, with all calves treated with the TSV-2 intranasal vaccine showing the greatest average daily weight gain.

TABLE O

Pen*	Treatment	Average Weight	Average Gain: 7-Day	14-Day	21-Day	28-Day	58-Day
25	Bulls Control	539.3	30.3	48.3	56.7	87.3	205.3
26	Steers Control	581.3	40.7	42.7	73.7	95.5	206.0
27	Bulls IM	541.8	24.8	43.8	58.2	111.3	245.7
28	Steers IM	596.2	16.2	27.8	67.2	94.5	210.7
29	Bulls TSV-2	540.3	29.3	43.0	74.0	103.2	223.5
30	Steers TSV-2	602.7	39.0	41.7	76.0	115.8	238.7

Average weight gains for calves vaccinated with IBR vaccine
*Six calves per pen.

TABLE P

Treatment	Average Daily Gain: 28-Day	58-Day	Gain as % Body Weight: 28-Day	58-Day
Controls	3.26	3.55	16.3	36.7
IM	3.68	3.93	18.1	40.1
TSV-2	3.91	3.98	19.2	40.4

Average Daily Gains for Each Test Group

EXAMPLE 12

The effect of bovine fibroblast IFN on the appetite of litter-mate piglets was examined. Seventy piglets were selected at birth for the test, with thirty-five piglets serving as controls. Each of the thirty-five piglets chosen to receive IFN treatment was orally administered 5 ml of a bovine IFN material with a titer of 7000 units/ml. The interferon was given on each of the three days before weaning. The weights of the piglets as monitored is shown in Table Q. The litter-mate piglets

differed in weight by only about 0.1 lb/pig at 21 days old, but 38 days after weaning, the IFN-treated pigs outweighed the controls by 1.5 lbs.

TABLE Q

No. of Pigs	Treatment	Effect of Bovine IFN on Weight of 70 Pigs: Weight (lbs.) at Day Birth	10	21	28	Weaning	W + 3	W + 10	W + 38
35	IFN	3.6	7.0	11.1	12.8	13.6	14.5	17.0	48.4
35	Controls	3.7	7.2	11.0	13.0	13.4	14.0	16.2	46.9
Benefit to IFN		−0.1	−0.2	+0.1	−0.2	+0.2	+0.5	+0.8	+1.5

EXAMPLE 14

Another study of the effect of bovine IFN on the weight gain of litter-mate piglets was conducted. Seventy-two piglets were used in the trial, thirty-six receiving interferon treatment with the remainder serving as controls. The IFN-treated pigs were each given 7 ml of bovine IFN with a titer of 7000 units/ml. The IFN was orally administered on each of the three days prior to weaning. The weights of the piglets are presented in Table R. Though the litter-mate piglets differed in weight by an average of only about 0.13 lb at 21 days old, the IFN-treated pigs outweighed the controls by an average of 1.70 lbs at 38 days after weaning.

TABLE R

No. of Pigs	Treatment	Effect of Bovine IFN on Weight of 72 Pigs: Weight (lbs.) at Day Birth	10	21	Weaning	W + 3	W + 10	W + 38
36	IFN	3.7	7.1	11.40	14.40	15.05	18.06	41.72
36	Controls	3.7	6.9	11.27	14.23	14.80	17.54	40.02
Benefit to IFN		0.0	+0.2	+0.13	+0.17	+0.25	+0.52	+1.70

The foregoing description has been directed to particular embodiments of the invention in accordance with the requirements of the Patent Statutes for the purposes of illustration and explanation. It will be apparent, however, to those skilled in this art that many modifications and changes in the apparatus and procedure set forth will be possible without departing from the scope and spirit of the invention. It is intended that the following claims be interpreted to embrace all such modifications and changes.

What is claimed is:

1. A method for increasing efficiency of food utilization of a warm-blooded vertebrate comprising administration to the warm-blooded vertebrate of a biologically active interferon in an amount effective to increase the efficiency in utilizing food for the warm-blooded vertebrate.

2. The method of claim 1 wherein the warm-blooded vertebrate is a mammal.

3. The method of claim 1 wherein the warm-blooded vertebrate is a bird.

4. The method of claim 1, 2, or 3 wherein the interferon is isolated from cells of bovine species origin.

5. The method of claim 4 wherein the interferon is fibroblast interferon.

6. A method for stimulating the appetite of cattle comprising administration to cattle an effective amount of a biologically active interferon found in cells of bovine species origin.

7. The method of claim 6 wherein the interferon is fibroblast interferon.

8. The method of claim 7 wherein each bovine is orally administered at least one dose of at least 10,000 units of bovine fibroblast interferon per kg of body weight.

9. The method of claim 7 wherein each bovine is intravenously administered daily for at least three days at least one dose of at least 4,000 units of bovine fibroblast interferon per kg of body weight.

10. A method for stimulating the appetite of cattle comprising inoculation of a bovine with a vaccinal virus strain in an amount effective to cause nasal secretion by the bovine of a biologically active interferon.

11. The method of claim 10 wherein the vaccinal virus strain is a vaccinal strain of infectious bovine rhinotracheitis virus.

12. The method of claim 11 wherein each bovine is inoculated with at least about 10^4 $TCID_{50}$ of such vaccinal strain.

13. A method for stimulating the appetite of swine comprising administration to swine an effective amount of a biologically active interferon found in cells of bovine species origin.

14. The method of claim 13 wherein the interferon is fibroblast interferon.

15. The method of claim 14 wherein the swine are piglets prior to weaning and the piglets are orally administered about 5,000 to about 50,000 units of bovine fibroblast interferon per kg of body weight per day for from one to five days before weaning.

16. A method for increasing the efficiency of food utilization in chickens comprising administration to chickens an effective amount of a biologically active interferon found in cells of bovine species origin.

17. The method of claim 16 wherein the interferon is fibroblast interferon.

18. The method of claim 17 wherein the chickens receive the bovine fibroblast interferon in their drinking water in an amount of from about 70 units to about 7,000 units per ml of drinking water.

* * * * *

Exhibit D

United States Patent [19]

Cummins, Jr.

[11] **Patent Number: 5,019,382**

[45] **Date of Patent: May 28, 1991**

A4

[54] **TREATMENT OF IMMUNO-RESISTANT DISEASE WITH LOW-DOSE INTERFERON**

[75] Inventor: **Joseph M. Cummins, Jr.**, Amarillo, Tex.

[73] Assignee: **The Texas A&M University System**, College Station, Tex.

[21] Appl. No.: **465,527**

[22] Filed: **Jan. 17, 1990**

Related U.S. Application Data

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[51] **Int. Cl.**[5] **A61K 37/66**

[52] **U.S. Cl.** **424/85.4**; 424/85.6; 424/85.7

[58] **Field of Search** 424/85.4, 85.6, 85.7

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[57] **ABSTRACT**

Neoplastic disease, hyperallergenicity, autoimmune disorders characterized by chronic tissue degenerative inflammation and immuno-resistant viral infections are treated by the administration of interferon at a dosage of about 0.1 to about 5 IU/lb per day by contacting said interferon with oral/pharyngeal mucosa. Interferon is administered in solution or in a novel solid unitary dosage form adapted to be dissolved in saliva when placed in the mouth.

14 Claims, No Drawings

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TREATMENT OF IMMUNO-RESISTANT DISEASE WITH LOW-DOSE INTERFERON

BACKGROUND OF THE INVENTION

This invention relates generally to an improved method of treating diseases of immuno-pathologic etiology in warm-blooded vertebrates using interferon in low oral dosages. This invention also relates to the use of interferon in low oral dosages to potentiate disease-corrective immune responses in warm-blooded vertebrates afflicted with immuno-resistant diseases characterized by apparent hyperactive or hypoactive immune system function.

"Interferon" is a term generically comprehending a group of vertebrate glycoproteins and proteins which are known to have various biological activities, such as antiviral, antiproliferative, and immunomodulatory activity at least in the species of animal from which such substances are derived. The following definition for interferon has been accepted by an international committee assembled to devise a system for the orderly nomenclature of interferons: "To qualify as an interferon a factor must be a protein which exerts virus nonspecific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein." *Journal of Interferon Research*, 1, pp. vi (1980). "Interferon" as used herein in describing the present invention shall be deemed to have that definition.

Since the first descriptions of interferon by Isaacs and Lindeman [See, *Proc. Roy Soc. London (Ser. B)*, Vol. 147, pp. 258 *et seq.* (1957) and U.S. Pat. No. 3,699,222], interferon has been the subject of intensive research on a worldwide basis. The literature is replete with publications concerning the synthesis of interferon, its proposed molecular characterizations, its clinical applications and proposed mechanisms of its antitumor, antiviral, and immune system activities.

Because of the intensity and disparate origins of research concerning interferon and its characteristics and uses, there exists a substantial lack of uniformity in such matters as classification of interferon types. There are also numerous, sometimes contradictory, theories concerning the mode of action of interferon in producing clinical effects.

Although originally isolated from cells of avian origin (chick allantoic cells), interferon production has been observed in cells of all classes of vertebrates, including mammals, amphibians, birds and reptiles. Interferon production by vertebrate cells is seldom spontaneous but is often readily "induced" by treatment of cells (in vivo or in vitro) with a variety of substances including viruses, nucleic acids (including those of viral origin as well as synthetic polynucleotides), lipopolysaccharides, and various antigens and mitogens.

Interferons have generally been named in terms of the species of animal cells producing the substance (e.g., human, murine, or bovine), the type of cell involved (e.g., leukocyte, lymphoblastoid, fibroblast) and, occasionally, the type of inducing material responsible for interferon production (e.g., virus, immune). Interferon has been loosely classified by some researchers according to induction mode as either Type I or Type II, with the former classification comprehending viral and nucleic acid induced interferon and the latter class including the material produced as a lymphokine through induction by antigens and mitogens. More recently, the international committee devising an orderly nomenclature system for interferon has classified interferon into types on the basis of antigenic specificities. In this newer classification, the designations alpha (α), beta (β), and gamma (γ) have been used to correspond to previous designations of leukocyte, fibroblast, and type II (immune) interferons, respectively. Alpha and beta interferons are usually acid-stable and correspond to what have been called type I interferons; gamma interferons are usually acid-stable and correspond to what has been called type II interferons. The international committee's nomenclature recommendations apply only to human and murine interferons. *Journal of Interferon Research*, 1 pp. vi (1980).

In its earliest applications, interferon was employed exclusively as an antiviral agent and the most successful clinical therapeutic applications to date have been in the treatment of viral or virus-related disease states. It became apparent, however, that exogenous interferon was sometimes capable of effecting regression or remission of various metastatic diseases. An overview of current clinical trials of interferon as an antiviral and antiproliferative therapeutic agent is contained in *Interferon: In Vivo and Clinical Studies*, Volume 4, Eds: N. B. Finter and R. K. Oldham, Academic Press, New York, 1985.

The clinical agent of choice for the present is human leukocyte interferon, "mass-produced" by procedures involving collection and purification of vast quantities of human buffy coat leukocytes, induction with virus, and isolation from culture media.

In the work described above, interferon has been administered parenterally, i.e., intramuscularly and intradermally, with some successful topical and intranasal usages having been reported. It has seldom been administered intravenously because of substantial adverse effects attributable to "contaminants" in crude and even highly purified isolates.

As discussed above, there has been a significant research effort directed to the evaluation of therapeutic effects of interferon for a wide variety of diseases having an auto-immuno-pathologic basis. Before applicant's first report of successful oral administration of interferon in his U.S. Pat. application Ser. No. 415,525 (now U.S. Pat. No. 4,462,985), there was no recognition in the art of the potential offered by oral administration of interferon. The generally held belief was that interferon could not survive the digestive conditions of the upper alimentary canal.

Since applicant's first disclosure of the immunotherapeutic benefit achievable via oral administration of interferon of heterologous mammalian species, he has continued to investigate the efficacy of orally administered interferon. In U.S. Pat. No. 4,497,795, issued Feb. 5, 1985, applicant described and claimed the use of interferon administered orally or via intravenous administration to stimulate appetite and feed efficiency of bovine and porcine species. More recently applicant has described in now pending U.S. applications the use of interferon at dosages less than about 5 IU per pound of body weight for increasing feed efficiency and food utilization in warm-blooded vertebrates, for preventing and treating shipping fever, and for enhancing vaccine efficiency.

Human alpha-interferon has been marketed under the trademark Agriferon ® by Immunomodulator Laboratories, Inc. ("IML") of Stafford, TX for veterinary use in Texas since February 1985. The product is sold for

oral administration to cattle to promote growth and feed efficiency and to prevent or treat viral respiratory infections. IML began selling an alpha-interferon product for horses in 1986. Both products are sold under a license of my U.S. Pat. 4,462,985.

SUMMARY OF THE INVENTION

Interferon contacting the oral and/or pharyngeal mucosa, in amounts of less than 5 IU/lb of body weight per day is consistently effective to potentiate disease-corrective immune responses in vertebrates afflicted with immuno-resistant disease states characterized by apparent hyperactive or hypoactive immune system function. Treatment in accordance with the present invention has been shown to effect remission of neoplastic disease, hyperallergenicity, immuno-resistant or immuno-debilitating viral infections and autoimmune disorders characterized by chronic tissue degenerative inflammation.

DETAILED DESCRIPTION OF THE INVENTION

The clinical agent of choice for use in the present invention is human leukocyte interferon (human alpha-interferon), "mass-produced" by procedures involving collection and purification of quantities of human buffy coat leukocytes, induction of interferon production with virus, and isolation of culture media. (See "Preparation of Human Alpha-Interferon" below.) Also acceptable for use in accordance with present intention are human alpha-interferon products produced by recombinant DNA technology and now commercially available from Schering-Plough (as Intron ®) and Hoffmann-LaRoche (as Roferon ®) and approved by the FDA for treatment (parenterally) of hairy cell leukemia of man. Such recombinant interferon products are believed to be particularly effective when used in combination. Gamma interferon is also available by recombinant technology and is presently undergoing clinical trials by Genentech and others. Fibroblast interferon (beta-interferon) can be prepared in accordance with Example 1 in applicant's U.S. Pat. No. 4,462,985 issued July 31, 1984, the disclosure of which is hereby expressly incorporated by reference.

Interferon of human and murine origins has been quantified in the art in terms of International Units ("IU"). As used herein, a "unit" of interferon (to be distinguished from "IU") shall mean the reciprocal of a dilution of interferon-containing material that, as determined by assay, inhibits one-half the number of plaques of a challenge virus, the challenge virus being the vesicular stomatitis virus ("VSV"). So quantified a "unit" of interferon is routinely found to be about one-tenth the quantity of interferon represented by one "IU." In other words, for the purpose of defining the present invention, 1 unit $\simeq$0.1 IU.

The present invention relates to an improved method of treatment of immuno-resistant disease states with interferon. The present invention is directed to the treatment of diseases in warm-blooded vertebrates, particularly certain diseases which the immune system of many species is poorly equipped to handle, as evidenced by either a lack of disease defeating response and/or an apparently misdirected immune response resulting in a chronic tissue degenerative inflammatory condition or other physical complications. While there has been a significant research effort directed to the use of interferon for treatment of such diseases, reported results, although positive overall, have been inconsistent. The principle reason for such inconsistency in view of my most recent research efforts is that earlier investigators have failed to define optimum dosage and route of interferon administration.

The present invention is based on applicant's discovery that interferon can be used as a consistently effective therapeutic agent for treatment of diseases having an immunopathologic basis—characterized by inadequate immune response and persistence of the disease or by an apparent hyperactive immune response resulting in tissue degenerative inflammatory conditions and related physical manifestations. Applicant has found that interferon, contacting the oral and pharyngeal mucosa in amounts from about 0.01 to about 5 IU/lb of body weight per day, is consistently efficacious for the treatment of diseases to which the immune system of many warm-blooded vertebrates does not effectively respond.

Disease conditions treated in accordance with the present invention include apparent autoimmune disorders characterized by a chronic tissue degenerative inflammatory condition. Diseases so characterized include multiple sclerosis, rheumatoid arthritis, stomatitis, and lupus erythematosus. Treatment of such disease is in accordance with the present invention comprises administering interferon at a dosage of 0.01 to about 5 IU/lb per day in a dosage form adapted to promote contact of said dosage of interferon with the oral and pharyngeal mucosa of said animal. Preferably, the dosage of interferon is from 0.1 to about 4.0 IU/lb per day, more preferably 0.5 to about 1.5 IU/lb of body weight per day. Alpha interferon, derived from tissue culture or by recombinant DNA techniques, is a preferred therapeutic agent in accordance with this invention. Alpha interferon can be administered alone or in combination with beta interferon or gamma interferon.

It is critical that the interferon be administered in a dosage form adapted to assure maximum contact of the interferon in said dosage form with the oral and pharyngeal mucosa of the human or animal undergoing treatment. Contact of interferon with the mucosa can be enhanced by maximizing residence time of the treatment solution in the oral or pharyngeal cavity. Thus, best results seem to be achieved in human patients when the patient is requested to hold said solution of interferon in the mouth for a period of time. Contact of interferon with the oral and pharyngeal mucosa and thereafter with the lymphatic system of the treated human or animal is unquestionably the most efficient method administering immunotherapeutic amounts of interferon.

Another disease condition responding to treatment in accordance with the present invention is neoplastic disease. Thus, the administration of interferon in accordance with the above description can, alone or in combination with other drugs or therapy, help effect remission of cancers such as malignant lymphoma, melanoma, mesothelioma, Burkitt lymphoma and nasopharyngeal carcinoma and other neoplastic diseases, especially those of known or suspected viral etiology. Based on the results observed to date, it is believed that applicant's presently described method of treatment will similarly help effect remission of Hodgkin's Disease and leukemia.

Other disease conditions responding to treatment in accordance with the present invention are infectious diseases of viral origin in, for example, human, avian, porcine, canine and feline species. Significantly, viral

infection typically exhibiting persistent resistance to treatment have shown a dramatic response to treatment with interferon in low doses contacting the oral and pharyngeal mucosa of infected patients. Beneficial results have been attained utilizing the present method to treat dogs having canine parvovirus and canine herpesvirus infections. Further, feline leukemia and feline infectious peritonitis have been shown to be particularly susceptible to treatment with alpha interferon and beta interferon in accordance with this invention.

Exemplary of human viral infections showing remarkable response to treatment in accordance with the present invention are infections of human rhinovirus (common cold), herpes simplex I virus (cold sores) and human papovavirus (warts). Based on treatment results to date, it is expected that contact of interferon at low dosage with the oral and pharyngeal mucosa will provide an effective treatment for Acquired Immune Deficiency Syndrome (AIDS) and disease conditions having the herpes simplex II virus as the causative agent. A patient experiencing a condition of viral myocarditis has responded favorably to the present treatment. Warts often dissipate within six to eight weeks after initiating treatment in accordance with this invention.

Other afflictions responding to contact of low dosage interferon are hyperallergenic conditions such as asthma. One "side effect" noted by patients treated in accordance with this invention is improved skin complexion. Thus, administration of interferon in dosages of about 0.01 to about 5 IU/lb of body weight per day is effective to treat acne, specifically and improve human skin complexion generally.

Further, stimulating the immune system by oral contact with low dosage interferon is believed to assist the body in fighting bacterial infection. Treatment in accordance with this invention alone or in combination with therapeutic amounts of antibiotics can be especially effective in knocking down infections of antibiotic resistant microorganisms.

Administration of interferon in accordance with the present invention is preferably continued until the symptoms of the disease condition being treated subside. This can range from a period of one day, for example, where a human rhinovirus is the disease causative agent, to a period of up to six months for treatment of neoplastic disease. Rheumatoid arthritis patients are pain free within 2 to 10 days of initiating treatment in accordance with the present invention. However, treatment of that disease is preferably conducted by administration of interferon for up to about three (3) months.

Daily dosage of interferon can be administered as a single dosage or, preferably, it is divided and administered in a multiple-dose daily regimen. A staggered regimen, for example one to three days treatment per week or month, can be used as an alternative to continuous daily treatment.

Interferon can be administered in accordance with this invention in either a liquid (solution) or solid dosage form. Thus interferon can be administered dissolved in a buffered aqueous solution typically containing a stabilizing amount (1–5% by weight) of blood serums. Exemplary of a buffered solution suitable as a carrier of interferon administered in accordance with this invention is phosphate buffered saline prepared as follows:

A concentrated (20x) solution of phosphate buffered saline (PBS) was prepared by dissolving the following reagents in sufficient water to make 1,000 ml of solution: sodium chloride, 160 grams; potassium chloride, 4.0 grams; sodium hydrogen phosphate, 23 grams; potassium dihydrogen phosphate, 4.0 grams; and optionally phenol red powder, 0.4 grams. The solution is sterilized by autoclaving at 15 pounds pressure for 15 minutes and then diluted with additional water to a single strength concentration prior to use.

Alternatively the interferon can be formulated into flavored or unflavored solutions or syrups using a buffered aqueous solution of interferon as a base with added caloric or non-caloric sweeteners, flavor oils and pharmaceutically acceptable surfactant/dispersants.

It is also contemplated by the present invention to provide interferon in a solid dosage form such as a lozenges adapted to be dissolved upon contact with saliva in the mouth with or without the assistance of chewing. Such a unitary dosage form is formulated to release about 1 to about 1500 IU of interferon upon dissolution in the mouth for contact with the oral and pharyngeal mucosa. Thus a unitary dosage form of interferon in accordance with this invention can be prepared by art-recognized techniques for forming compressed tablets such as chewable vitamins. Similarly, interferon can be incorporated into starch-based gel formulations to form a lozenge which will dissolve and release interferon for contact with the oral mucosa when held in the mouth. Solid unitary dosage forms of interferon for use in accordance with the present invention can be prepared utilizing art recognized dosage formulation techniques. The pH of such formulations can range from about 4 to about 8.5. Of course, in processing to such unitary dosage forms one should avoid heating a pre-dosage form formulation, after addition of interferon, above about 50° Centigrade.

Preparation of Human Aloha-Interferon

Human alpha-interferon can be prepared through the following procedure, commonly referred to as the Cantell procedure. The process begins with packs of human leukocytes, obtained in this case from the Gulf Coast Regional Blood Center, Houston, Texas. The buffy coats in these packs are pooled into centrifuge bottles, and then are diluted with 0.83% ammonium chloride. The mixture is incubated for 15 minutes with intermittent shaking, and is then centrifuged for 20 minutes at 2000 rpm. The supernatant is discarded, and the cell pellets are resuspended with a minimal volume of sterile phosphate buffered saline (PBS). The mixture is then diluted with ammonium chloride and centrifuged. The supernatant is again discarded, and the remaining cell pellets are resuspended with a minimal volume of a tissue culture medium such as Minimal Essential medium (MEM), available from KC Biological. The cell concentration is determined with a Coulter counter.

Interferon induction takes place in glass or plastic bottles. The induction medium contains MEM 75 mM Hepes (available from Calbiochem), 75 mM Tricine (available from Sigma Chemical Co.), human agamma serum (18 mg/ml), and gentamycin sulfate (from M.A. Bioproducts; 50 mcg/ml). The cells are added to the induction vessels at a final concentration of about 5 to 10 million cells per milliliter. The induction vessel is incubated in a 37° C. water bath, and interferon alpha is added as a primer.

After two hours, Sendai virus is added to the induction mixture. This causes alpha interferon to be produced in the supernatant by the leukocytes. After a 12–18 hour incubation time, the induction mixture is

centrifuged. The cells are discarded, and the supernatant is then purified.

The crude interferon is chilled to 10° C. or below in an ice bath. Five molar potassium thiocyanate is added to obtain a final concentration of 0.5M. This solution is stirred for 15 minutes, and then its pH is lowered to 3.3 by adding hydrochloric acid. The mixture is then centrifuged at 2800 rpm for 30 minutes, and the supernatant is discarded.

The pellets are then resuspended in 95% ethanol and are stirred for 15 minutes. This suspension is centrifuged at 2800 rpm for 20 minutes, and the pellets are discarded. The pH of the supernatant is then adjusted to 5.8 with sodium hydroxide. The mixture is stirred for 10 minutes, and then centrifuged at 2800 rpm for 20 minutes. The pellets are discarded. The pH of the supernatant is then adjusted to 8 with sodium hydroxide. This solution is stirred for 10 minutes, followed by centrifugation at 2800 rpm for 20 minutes. The supernatant is discarded, and the pellets are resuspended with 0.5M potassium thiocyanate in a 0.1M sodium phosphate buffer. This suspension is stirred at 4° C.

Next, the suspension is centrifuged at 2800 rpm for 20 minutes, and the pellets are discarded. The pH of the supernatant is adjusted to 5.3 with hydrochloric acid. After stirring for 10 minutes and centrifugation, the pH of the supernatant is adjusted to 2.8 with hydrochloric acid, followed by further stirring for 20 minutes. This mixture is centrifuged at 2800 rpm, and the resulting pellet is purified human alpha-interferon.

The pellet is resuspended with 0.5M potassium thiocyanate in 0.1M sodium phosphate buffer, having a pH of 8.0. It is then dialyzed against PBS at 4° C., with two changes of PBS. This mixture is then centrifuged and the precipitate is discarded. The remaining purified alpha interferon is sterilized by filtration through a 0.2 micron filter. A human alpha-interferon is produced in accordance with this procedure by Immuno Modulators Laboratories, Inc., Stafford, TX, and sold under the trademark Agriferon ® for use in cattle and Equiferon ® for use in horses.

Other procedures known to those skilled in the art are available for making interferons, such as human alpha-interferon and human gamma-interferon. For example, U.S. Pat. Nos. 4,376,821 and 4,460,685 disclose methods of making human gamma-interferon. A method of making bovine fibroblast (beta) interferon is disclosed in applicant's U.S. Pat. No. 4,462,985.

Clinical Studies

Tables 1-4 below summarize the results of clinical studies of the administration of interferon by veterinarians orally to 137 dogs and cats as of Nov., 1985. The studies were conducted with both human alpha-interferon and bovine beta-interferon. Tables 1-4 compare survival rates of pets with feline leukemia virus-associated diseases or canine parvovirus disease. Unequal numbers of pets were treated with each type of interferon; bovine beta-interferon was given to 78 pets and human alpha-interferon was given to 59 pets.

Bovine beta-interferon was produced in flasks of confluent monolayers of bovine fetal kidney (BFK) cells. Culture supernatant was harvested 24 hours after bluetongue virus induction of BFK cells. The supernatant was dialyzed 24 hours in a pH 2.0 buffer and for another 24 hours in a PBS (pH 7.4) buffer before interferon assay. Procedures for the assay and characterization of bovine beta-interferon were essentially as described by Rosenquist and Loan, *American Journal of Veterinary Research*, 28; 619-628, 1967. Interferon titers as "units" were expressed as the reciprocals of the dilutions that provided a 50% reduction in the number of VSV plaques as compared with the number in control cultures. The BFK cell culture interferon produced by this method had an average titer of 7,000 units per milliliter. Dogs were given bovine beta-interferon, 5-10 ml/dose, as least three times/day after a diagnosis of CPV disease. Cats positive by ELISA for feline leukemia virus and exhibiting clinical signs of disease were given 1 ml/10 lb of body weight 2-3 times daily for five days. After a five-day interval, cats were retreated at least once for another five days.

Human alpha interferon was obtained from IML, Inc. of Houston, TX. Cases were treated with lot AO26 applied at 6×10^6 IU/ml. Lot AO26 of human alpha interferon was diluted 1:150 in Eagles' minimum essential medium (MEM) and used as the stock solution from which 1 ml was further diluted 1:1000 with 1 liter of MEM for treatment. The usual dose of human alpha interferon was 4 IU/lb body weight given at least three times daily after a diagnosis of CPV disease was made. For feline leukemia, cats were treated with human alpha-interferon 2-3 times daily for five days as reported for bovine beta-interferon.

Significantly ($P<0.05$) more cats lived six and twelve months after diagnosis and treatment for feline leukemia virus if alpha-interferon was given, compared to treatment with bovine beta-interferon. Significantly ($P<0.05$) more dogs survived CPV disease when given alpha interferon (92%) compared to those dogs given bovine beta-interferon (69%).

TABLE 1

Summary of Survival Date from clinically ill cats positive for FeLV.

	Months After Treatment		
Treatment	1	6	12
Human alpha-IFN	25/33	21/32*	19/31*
Bovine IFN	26/36	15/36	13/36

Numerator = no. alive; denominator = no. treated.
*Cats given human alpha-IFN had significantly ($P < .05$) higher survival rates at 6 and 12 months after treatment than cats given bovine IFN. Significance was determined by Chi Square test.

TABLE 2

Percent survival of clinically ill cats positive for FeLV.

	Months After Treatment		
Treatment	1	6	12
Human alpha-IFN	76%	66%*	61%*
Bovine IFN	72%	42%	36%
Historical Control	<50%	<30%	—

Numerator = no. alive; denominator = no. treated.
*Cats given human alpha-IFN had significantly ($P < .05$) higher survival rates at 6 and 12 months after treatment than cats given bovine IFN. Significance was determined by Chi Square test.

TABLE 3

Response of CPV disease to treatment with bovine interferon or human alpha-interferon, by veterinarian.

Attending	Bovine IFN Beta		Human Alpha IFN	
Veterinarian	Lived	Died	Lived	Died
S	16/21	5/21	14/16	2/16
M	6/11	5/11	7/7	0/7
R	7/10	3/10	3/3	0/3

TABLE 3-continued

Response of CPV disease to treatment with bovine interferon or human alpha-interferon, by veterinarian.

Attending	Bovine IFN Beta		Human Alpha IFN	
Veterinarian	Lived	Died	Lived	Died
	29/42(69%)	13/42(31%)	24/26(92%)	2/26(8%)

Dogs treated with human alpha-interferon had a significantly ($P < .05$) higher survival rate compared to dogs treated with bovine IFN. Significance between groups was determined by Chi Square test.

TABLE 4

Treatment days for CPV disease.

Treatment	No. of Days	Average No. Treatment Days*	SD**	Survival Rate
Bovine IFN	42	3.31	1.95	69%
Human alpha IFN	26	2.75	0.92	92%

*Calculated on surviving dogs only.
**Standard deviation of the mean treatment days.

Canine Heroesvirus Challenge of Newborn Dogs

Canine herpesvirus infection of dogs less than one week of age are invariably fatal, but older pups usually survive. Interferon has been successfully used to treat viral infections of many species. These studies were conducted to assess the efficacy of interferon in canine herpesvirus (CHV) inoculated puppies.

Five (5) pregnant bitches were obtained from a USDA licensed supplier and were housed in a USDA approved research facility in Canyon, Texas. After the pups whelped, they were inoculated with 6.3 log 10 units of virulent CHV obtained from Dr. Richard Mock of the Texas A&M University Veterinary Medical Diagnostic Laboratory (TVMDL) in Amarillo, Texas. Human alpha-interferon (HAI) or placebo was given to pups orally as treatment in an effort to increase the survival rate of the CHV inoculated pups. Each litter was divided into control and treated animals. The procedures and schedule for each litter are discussed below. All dead animals were necropsied at TVMDL, Amarillo, TX.

LITTER 1

Nine (9) pups were inoculated orally with CHV on the day of birth. Interferon was given at 6–10 units (1X), or ten times the dosage at 60–100 units (10X). Three pups were given 0.5 ml placebo, 3 pups were given 0.5 ml HAI (1X), and 3 pups were given 0.5 ml of a 10X concentrate of HAI orally twice daily for 7 days (if they lived that long). The 3 controls died 5, 6, and 8 days after CHV inoculation. The 3 pups given HAI (1X) lived 7, 7, and 9 days and the 3 pups given 10X HAI lived 6, 7, and 7 days after CHV inoculation.

Pups given HAI (1X) lived an average of 1.3 days longer than controls, but the longer survival time was not statistically significant. The higher dosage, HAI (10X), did not provide a survival benefit over the lower dosage, but instead pups given the higher dosage died, on the average, one day sooner.

LITTER 2

Eight (8) pups were inoculated with CHV orally 2 days after birth. Interferon was given at 6–10 units (1X), or ten times the dosage (10X), or 1/10th the dosage (1/10X). All interferon was given orally after dilution in PBS. Two (2) pups were given 0.5 ml PBS, 2 were given 0.5 ml HAI at 1/10th concentration, 2 were given HAI (1X), and 2 were given a 10X concentrate of HAI. All treatments were given orally twice daily for 5 days starting 1 day before CHV inoculation. The 2 controls died <1 and 9 days after CHV inoculation and the HAI treated (1/10th dose, full dose, 10X dose) pups died 8 and 9, 5 and 9, 8 and 8 days after CHV inoculation, respectively.

No benefit from treatment at any dosage was seen. The death of a control pup within a day after CHV inoculation was probably not related to CHV inoculation.

LITTER 3

Nine (9) pups were inoculated with CHV orally 3 days after birth. Two pups were given 0.5 ml PBS, 2 were given 0.5 ml HAI (1X), 2 were given 1/10th dose HAI, and 3 were given 2 IU of recombinant human alpha-interferon from Schering-Plough. All treatments were given orally twice daily for 5 days starting two days before CHV inoculation. Both pups given HAI (1X) survived until necropsied 19 days after CHV inoculation. One control pup died 14 days after CHV inoculation and 1 survived until necropsied 19 days after CHV inoculation. Pups given 1/10th dosage of HAI died 8 and 13 days after CHV inoculation. Only one pup given recombinant human alpha-interferon died (12 days after CHV); the other 2 pups survived until necropsied 19 days after CHV inoculation.

These pups, inoculated 3 days after birth, did not develop an overwhelming CHV infection (only 1 of 2 controls died). A 1/10th dose of HAI did not protect either pup but both HAI (1X) treated pups survived.

LITTER 4

Fourteen (14) pups were inoculated orally with CHV 2 days after birth. Seven (7) pups were given PBS and 7 pups were given HAI (1X) orally twice daily for 7 days (if they lived that long) starting 2 days after CHV inoculation. The 7 controls died 1, 5, 7, 8, 8, 9, and 9 days after CHV inoculation. One of the HAI (1X) treated pups survived and the other 6 pups died 1, 6, 8, 9, 9, and 12 days after CHV inoculation. The deaths of 2 pups only 1 day after CHV inoculation were probably not related to CHV inoculation.

One HAI (1X) treated pup lived 3 days beyond the last surviving control and one HAI (1X) treated pup lived 2 weeks (until necropsied) beyond any treated control pup. Average survival time of interferon treated pups was longer than control survival time, but not significantly so.

LITTER 5

Six (6) pups were inoculated orally with CHV 2 days after birth. Three (3) pups were given PBS and 3 were given HAI (1X) orally once daily starting 5 days after CHV inoculation. The 3 controls died 6, 6, and 7 days after CHV inoculation. One of the HAI (1X) treated pups survived (until necropsied) and the others died 8 and 9 days after CHV inoculation.

All interferon treated pups lived longer than any of the control pups. Treatment with HAI (1X) did not begin until 5 days after CHV inoculation, yet survival was significantly ($P<0.05$) prolonged.

In summary, on the average, puppies treated with human alpha-interferon had longer survival times and enhanced survival rates compared to littermate controls, after canine herpesvirus challenge. A total of 7 puppies (1 control and 6 interferon treated) survived the normally fatal CHV inoculation. The data is summarized in the Table 5 below.

TABLE 5

Summary of Canine Herpesvirus Data

Litter	No. of Pups	Dosage	Average* Survival Time (Days)	Survivors
1	3	control	6.33	0
1	3	HAI 1X	7.67	0
1	3	HAI 10X	6.67	0
2	2	control	4.5**	0
2	2	HAI 1/10th	8.5	0
2	2	HAI 1X	7.0	0
2	2	HAI 10X	8.0	0
3	2	control	14.0	1
3	2	HAI 1/10th	10.5	0
3	2	HAI 1X	—	2
3	3	recombinant IFN	12.0	2
4	7	control	6.7	0
4	7	HAI 1X	7.5	1
5	3	control	6.3	0
5	3	HAI 1X	8.5	1

*dead dogs survival time; living puppies not calculated.
**includes one pup living only one day beyond CHV inoculation.

Treatment Of Nasal Solar Dermatitis

Three cases of nasal solar dermatitis (collie nose) cleared after human alpha-interferon treatment of 1 unit/lb body weight orally and topical treatment (a few ml at 20 units/ml).

Treatment of Canine Lupous Erythematosus

Two cases diagnosed as canine lupus erythematosus were cured by human alpha-interferon treatment. A 2 year-old Lhasa apso male had been treated with prednisolone for 1 year for 3 dermatological lesions on the abdomen and prepuce. The flat glistening lesions were continually licked by the dog. Within 1 week of oral human alpha-interferon treatment (1 unit/lb body weight daily for 5 days, then after 1 week, treatment was repeated for 5 days) 2 lesions completely healed and the third lesion was reduced to ½ its original size. Within 4 weeks, the lesions were all completely healed and all therapy ceased. One year later, a skin lesion reappeared but promptly healed after interferon treatment was repeated. The skin lesions have not reappeared in the past 10 months.

A 6 year-old spayed female chihuahua cross had a spider shaped (4 cm by 2 cm approximately) skin lesion on the abdomen. The lesion was flat, glistening and pruritic. Six weeks of prednisolone treatment resulted in complete healing. The following summer, the lesion reappeared and was treated with oral human alpha-interferon at about 1 unit/lb body weight daily for5 days. Within 5 days the lesion was reduced to ⅓ its original size and completely disappeared within 10 days. The lesion has not reappeared in the past year.

Treatment Of Feline Infectious Peritonitis

Table 6 shows the results of 17 cases of feline infections peritonitis (FIP) as diagnosed by practicing veterinarians. Human alpha-interferon (IFN) treatment resulted in a significantly greater survival rate than treatment with bovine beta-IFN.

TABLE 6

Survival of clinically ill cats diagnosed as FIP

Treatment	No. Cats Treated	Alive	Dead	Survival Rate
Human alpha-IFN	11	10	1	91%
Bovine beta-IFN	6	3	3	50%
Total	17	13	4	76%

Cats given human alpha-IFN had a significantly (P = .0574) greater survival rate than cats given bovine beta-IFN.

Human Treatment With Exogenous Human Lapha-Interferon

Human patients were treated with human alpha-interferon in the therapy of acute rheumatoid arthritis, multiple sclerosis, astham, acne, malignant lymphoma, mesothelioma, and apthous stomatitis. Therapy consisted of oral administration of 0.7 IU per lb. of patient body weight twice daily, once in the morning and once in the evening. None of the patients noted any fever or anorexia associated with the administration of alpha interferon. Interferon was administered in a buffered solution having a concentration such that a single dosage could be administered in a volume of about 1 to about 20 ml of liquid. Each patient generally retained the interferon solution in his mouth for a period of time up to about one minute. After that time the solution was either swallowed or discharged from the patient's mouth.

Two patients suffering from rheumatoid arthritis were treated—a Caucasian male age 44 and a Caucasian female age 44. The male patient was pain free in 7 days, and the female was pain free in 10 days. They were both continued on the oral interferon for 21 days total and have remained asymptomatic.

It has been found that recurrence of a treated arthritic condition can be minimized if treatment in accordance with the present invention is continued over a period of up to about three months.

A 30-year-old Caucasian female nurse afflicted with multiple sclerosis and who had an extensive neurologic workup at City of Hope Hospital in Los Angeles received treatment in accordance with the present invention for 21 days. The patient has had no recurrence of her neurologic symptoms for the past nine months.

A 42-year-old Caucasian male diagnosed to have a malignant lymphoma had completed chemotherapy with dismal results and was considered terminal. He was treated for three weeks with oral interferon. Six months after starting treatment he was released by his oncologist as free of the disease.

An 82-year-old Caucasian female was diagnosed to have mesothelioma. Presently there is no effective treatment for that disease and only a 9-month average survival rate is predicted. During her treatment with human alpha-interferon she had thoracentesis on two occasions for plural effusion. Otherwise, the patient has been active and has survived for 43 months.

A 32-year-old Asian male with apthous stomatitis was treated for two weeks with human alpha-interferon in accordance with the present invention. There has been no recurrence of the ulcers over the last six months since treatment was completed.

BKC is a 29 year-old Caucasian female and KKJ is a 20 year-old Caucasian female. Both are afflicted by acne-like skin blemishes at the time of their monthly menstrual cycle. Oral human alpha-interferon given at about 1 unit/lb of body weight for 3 days prior to the

time of their cycle reduces the severity and number of skin blemishes.

Treatment Of Warts In Humans

With Bovine Aloha-Interferon

MAH, a 38 year-old Caucasian female, had 7 warts on the middle finger of her right hard. After 9 months duration, medical treatment was sought, and liquid nitrogen was applied by a dermatologist. Only one wart on the finger regressed after treatment. Three warts coalesced to create a large wart area that, over the next year, acquired a roughly 12 millimeter square shape. Oral bovine alpha interferon treatment was started at a dosage of 6 ml daily for 6 consecutive days. The concentration of alpha-interferon was 30 units/ml; it was derived from the nasal secretions of cattle infected with infectious bovine rhinotracheitis virus. All the warts completely regressed within 6 weeks of the first dose of interferon.

Interferon Dosage Formulations

(1) Lozenge

A starch gel-based lozenge containing interferon is prepared by combining 150 grams of sucrose, 550 ml phosphate buffered saline, and 250 grams of a cold-water-soluble starch such as that described in U.S. Pat. No. 4,465,702, heating that mixture with stirring to a temperature of about 75° C., cooling the mixture to about 30° C. and thereafter blending into the paste-like mass 50 ml of phosphate buffered saline PBS containing human alpha interferon at a concentration of 250 IU/ml. The mixture is then formed into multiple portions of about 5 to about 10 grams each which set upon standing under drying conditions to a starch candy gel-like consistency. The lozenges thereby produced can be administered to a patient singly or in combination. The patient is instructed to hold the lozenge in his mouth until it is completely dissolved to release the interferon component for contact with the oral mucosa.

(2) Chewable Vitamin

A chewable vitamin formulation is prepared, for example, according to the description of U.S. Pat. No. 3,857,939 by coating one or more components thereof prior to tableting with an interferon solution in an amount sufficient to provide about 1 to about 1500 units of interferon in each chewable vitamin tablet.

(3) Mouthwash

A mouthwash formulation is prepared in accordance with the present invention by combining 850 0 ml PBS, 100 ml of glycerin, 50 grams of dextrose, and a mixture of 0.3 ml of a flavor oil pre-mixed with 30 ml of a palatable, pharmaceutically acceptable surfactant/dispersant having an HLB from about 15 to about 25 and 50 ml of a PBS solution of interferon (concentration 120 IU/ml). The formulation contains interferon at a concentration of about 120 IU per 20 ml dosage. The patient is asked to hold a 20 ml volume of the mouthwash in his mouth, optionally gargling with the same, for a period of about 15 seconds to about one minute.

(4) Syrup

Interferon is added to a commercial cough syrup formulation in an amount sufficient to provide an interferon containing syrup formulation having about 1 to about 1500 IU of human interferon per tablespoon of syrup.

(5) Effervescent Tablet

A tableting mixture comprising a pharmaceutically acceptable alkali metal carbonate or bicarbonate, an organic acid such as citric acid, human interferon (preferably dispersed on a suitable organic or inorganic carrier therefor) in an amount sufficient to provide a per tablet dose of about 1 to about 1500 units of interferon per dose, and further including suitable tableting excipients such as lubricants and binders, is compressed into a unitary dosage form of interferon. The compressed tablet effervesces upon contact with water to release interferon to the resulting buffered solution. The dosage of interferon is readily available in solution for contact with the oral pharyngeal mucosa of a patient in need of said dosage of interferon.

I claim:

1. An improved method of treatment of infectious disease of viral origin in human, canine and feline species said method consisting essentially of contacting about 0.01 to about 5 IU of interferon/lb of body weight per dose with the oral and pharyngeal mucosa of said species.

2. The method of claim **1** wherein the dosage is about 0.1 to 4.0 IU/lb per day.

3. The method of claim **1** wherein the interferon is selected from alpha-interferon and beta-interferon.

4. The method of claim **1** wherein the interferon is alpha-interferon produced from human leukocytes.

5. The method of claim **1** wherein human alpha-interferon is administered at dosage of about 0.5 to 1.5 IU/lb of body weight per day.

6. The method of claim **5** wherein a human is treated for a viral infection selected from the group consisting of human rhinovirus, herpes simplex I virus, herpes simplex II virus, viral myocarditis and HILV III virus (AIDS).

7. The method of claim **1** wherein a human is treated for warts.

8. The method of claim **1** wherein a feline species is treated for feline leukemia virus or feline infectious peritonitis.

9. The method of claim **3** wherein a canine species is treated for canine parvovirus.

10. The method of claim **1** wherein canine species is treated for canine herpesvirus.

11. The method of claim **1** wherein a human suffering from Acquired Immune Deficiency Syndrome is treated with human alpha-interferon or human beta-interferon.

12. The method of claim **11** wherein about 0.5 to about 1.5 of interferon/lb of body weight in aqueous solution is held in the patient's mouth for a period of time sufficient to allow contact of the interferon with the oral mucosa of said patient.

13. The method of claim **6** wherein the dosage of interferon is divided and administered as a multiple dose daily regimen.

14. The method of claim **11** wherein beta-interferon is administered at a dosage of about 0.5 to about 1.5 IU/lb per day.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,019,382

DATED : May 28, 1991

INVENTOR(S) : Joseph M. Cummins, Jr.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, under "References Cited", the following references should be added:

Under "U.S. Patent Documents":

4,803,072	2/1989	Dalton et al
4,828,830	5/1989	Wong

Under "Foreign Patent Documents":

PCT/DK82/00092	4/1983

Column 6, line 35, change "Aloha-Interferon" to --Alpha-Interferon--.

Column 9, line 20, change "Heroesvirus" to --Herpesvirus--.

Column 11, line 28, change "Lupous" to --Lupus--.

Column 12, line 12, change "Lapha-Interferon" to --Alpha-Interferon--.

Column 13, line 5, change "Aloha-Interferon" to --Alpha-Interferon--.

Column 14, line 37 (claim 6), change "HILV" to --HTLV--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,019,382

DATED : May 28, 1991

INVENTOR(S) : Joseph M. Cummins, Jr.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 14, line 53 (claim 12), after "1.5", insert --IU--.

Signed and Sealed this

Eighth Day of September, 1992

Attest:

DOUGLAS B. COMER

Attesting Officer

Acting Commissioner of Patents and Trademarks

XI. RELATED PROCEEDINGS APPENDIX

There are no related proceedings to this action, thus no copies of proceedings are provided.